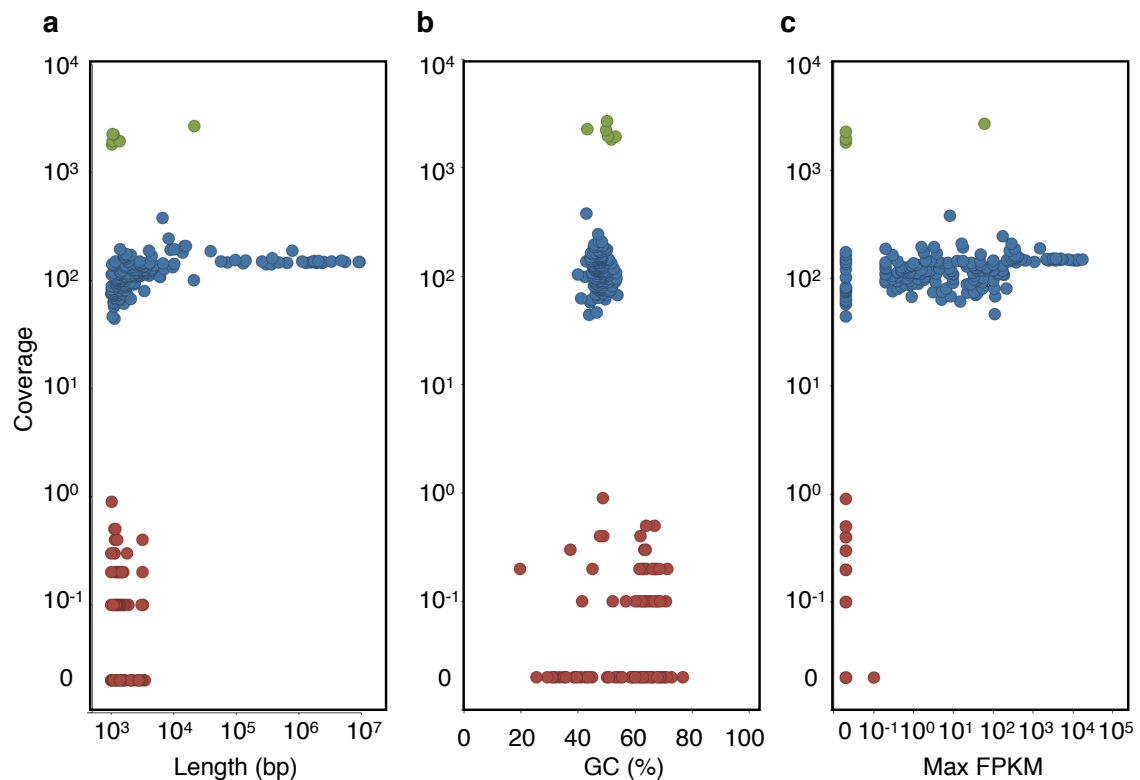
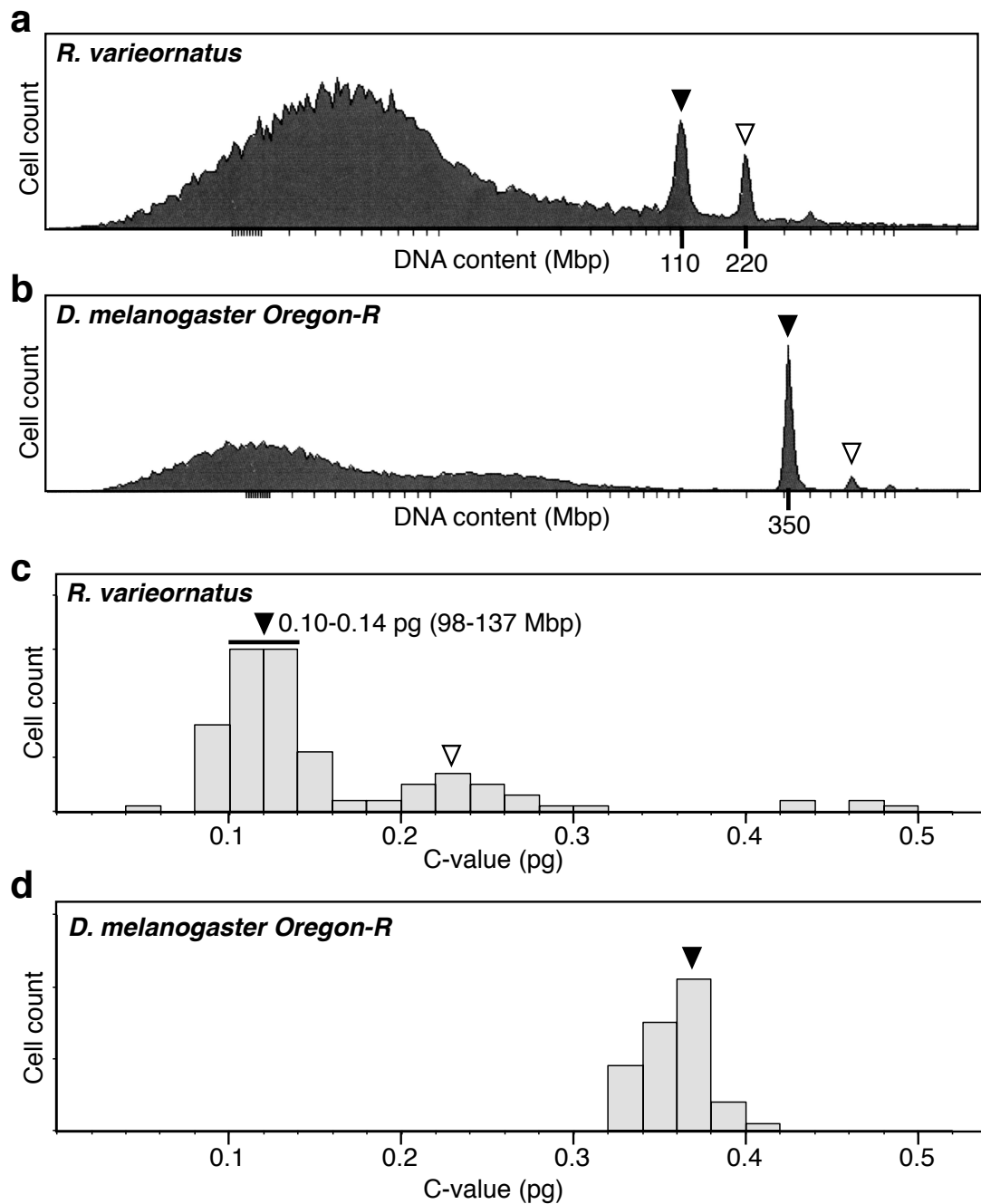


## Supplementary Figures



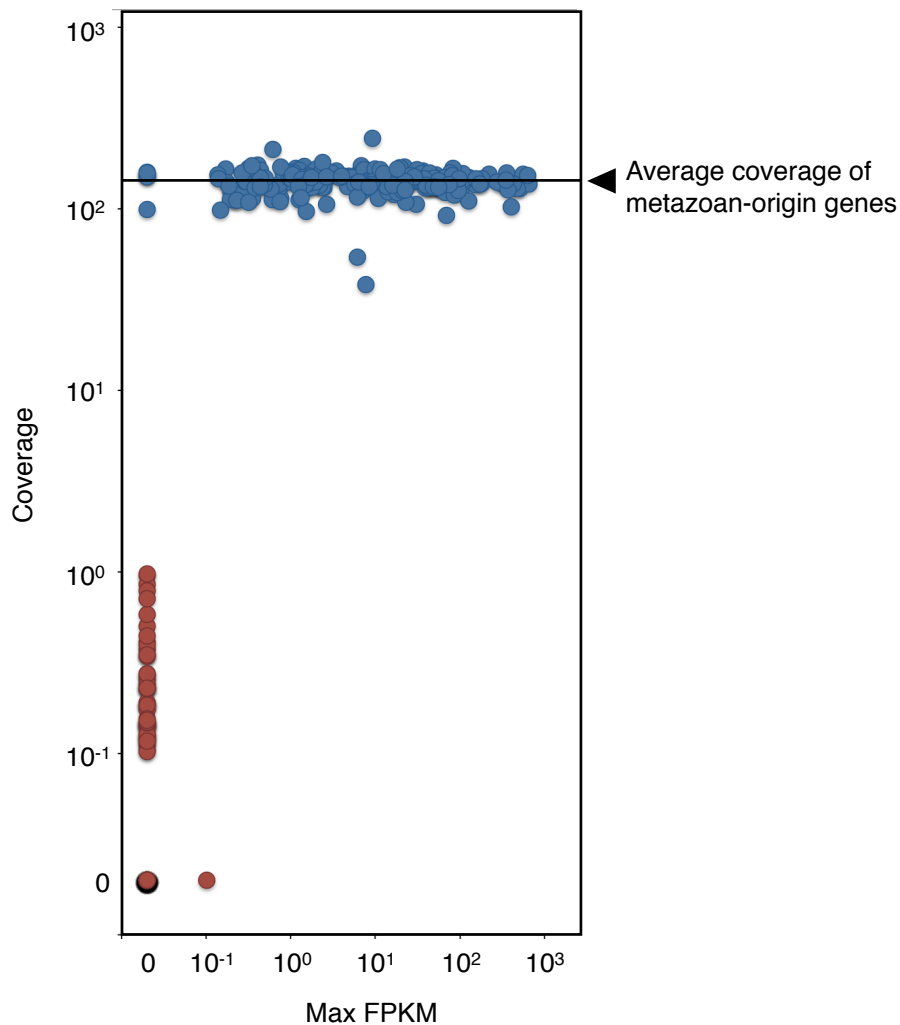
### Supplementary Figure 1. Classification of scaffolds in unfiltered genome assembly

(a) Distribution of scaffolds by length and coverage with Illumina genomic reads (160x). Green spots indicate scaffolds harbouring rDNA sequences. Red spots indicate scaffolds with low coverage (<1), classified as putative contaminating sequences. Blue spots indicate scaffolds with moderate coverage (approximately 40-400). Scaffolds indicated by blue and green spots, were included in the final genome assembly. (b) Distribution of scaffolds by GC proportion and coverage. Blue and green spots exhibited consistent GC proportion, whereas red spots distributed in wide range of GC proportions. (c) Distribution of scaffolds by maximum gene expression of protein-coding genes (FPKM) and coverage. Genes encoded in red scaffolds exhibited no or quite low expression in transcriptome analyses.



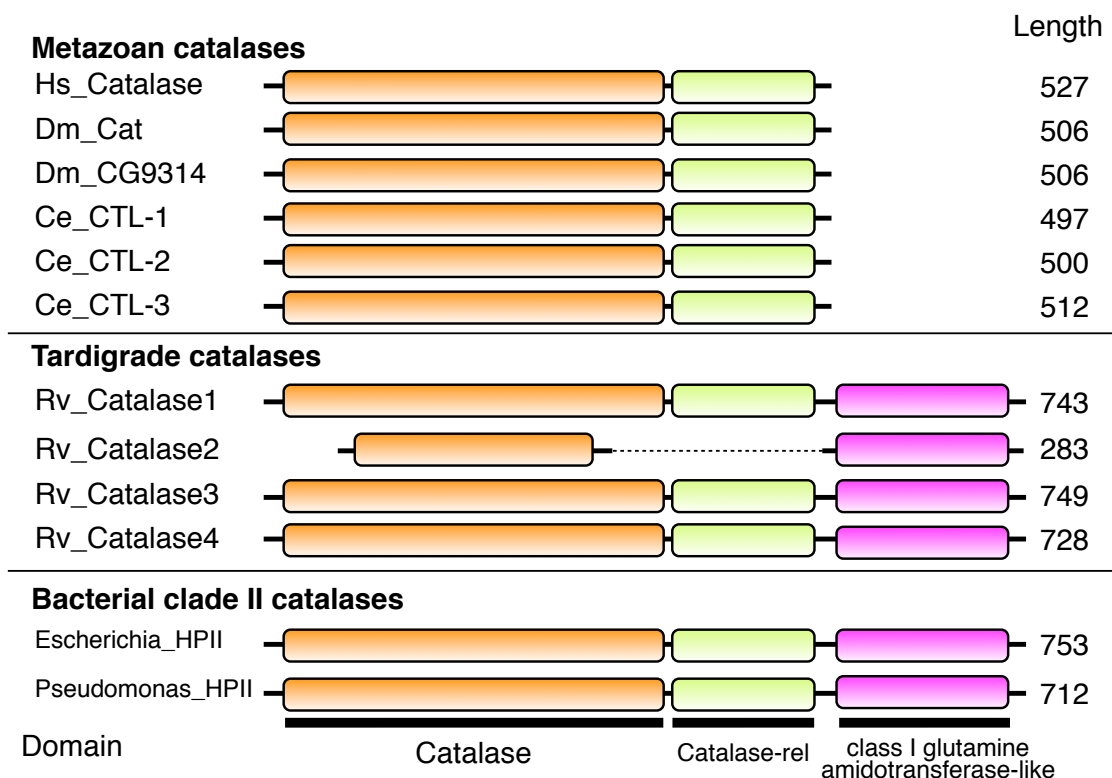
**Supplementary Figure 2. Genome size determination of *R. varieornatus***

(a, b) Genome size of *R. varieornatus* was estimated by propidium iodide staining and flow cytometry. *D. melanogaster* cells were used as a reference for quantification. Black and white arrowheads indicate peaks corresponding putative diploid (2C) and tetraploid (4C) cells. DNA content of the putative diploid cells of *R. varieornatus* was estimated as 110 Mbp. (c, d) Genome size estimation by Feulgen densitometry. The peak of putative diploid cells in *R. varieornatus* was relatively broad and the DNA content was estimated in the range of 98-137 Mbp, which was well consistent with the estimation by flow cytometry.



### Supplementary Figure 3. Genomic read coverages and expressions of putative HGT genes

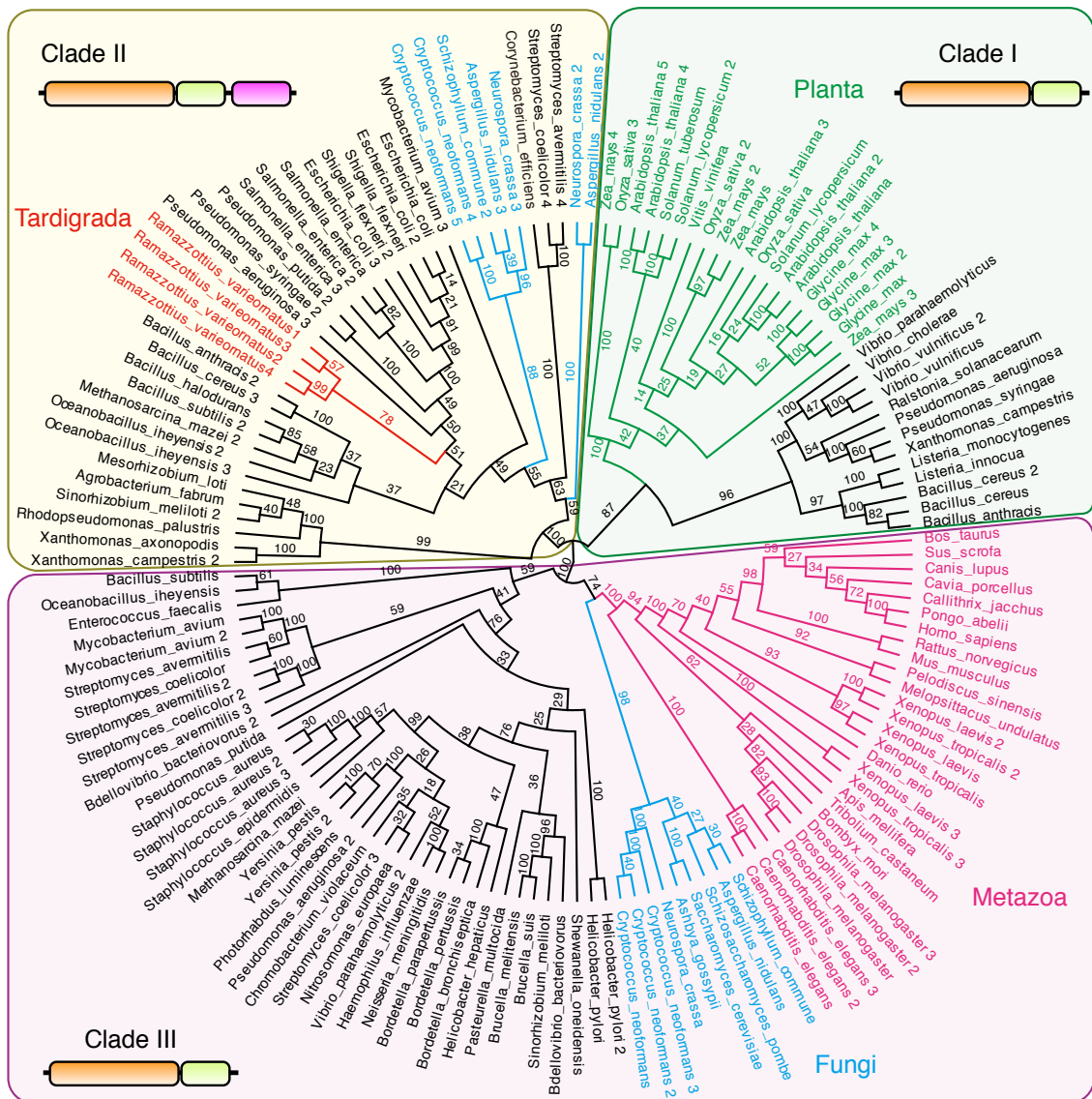
Distribution of putative HGT genes by maximum gene expression (FPKM) and coverage with genomic reads. Blue spots indicate 234 putative HGT genes encoded in the scaffolds involved in the final assembly. Most of them exhibited substantial coverage with genomic reads (ranging from 38 to 245) comparable to those in metazoan-origin genes (149 in average; ranging from 27 to 600). Red spots indicate 129 putative HGT genes encoded in the putative contaminating scaffolds, which were excluded from the final assembly.



**Supplementary Figure 4. Schematic comparison of domain structure of catalases**

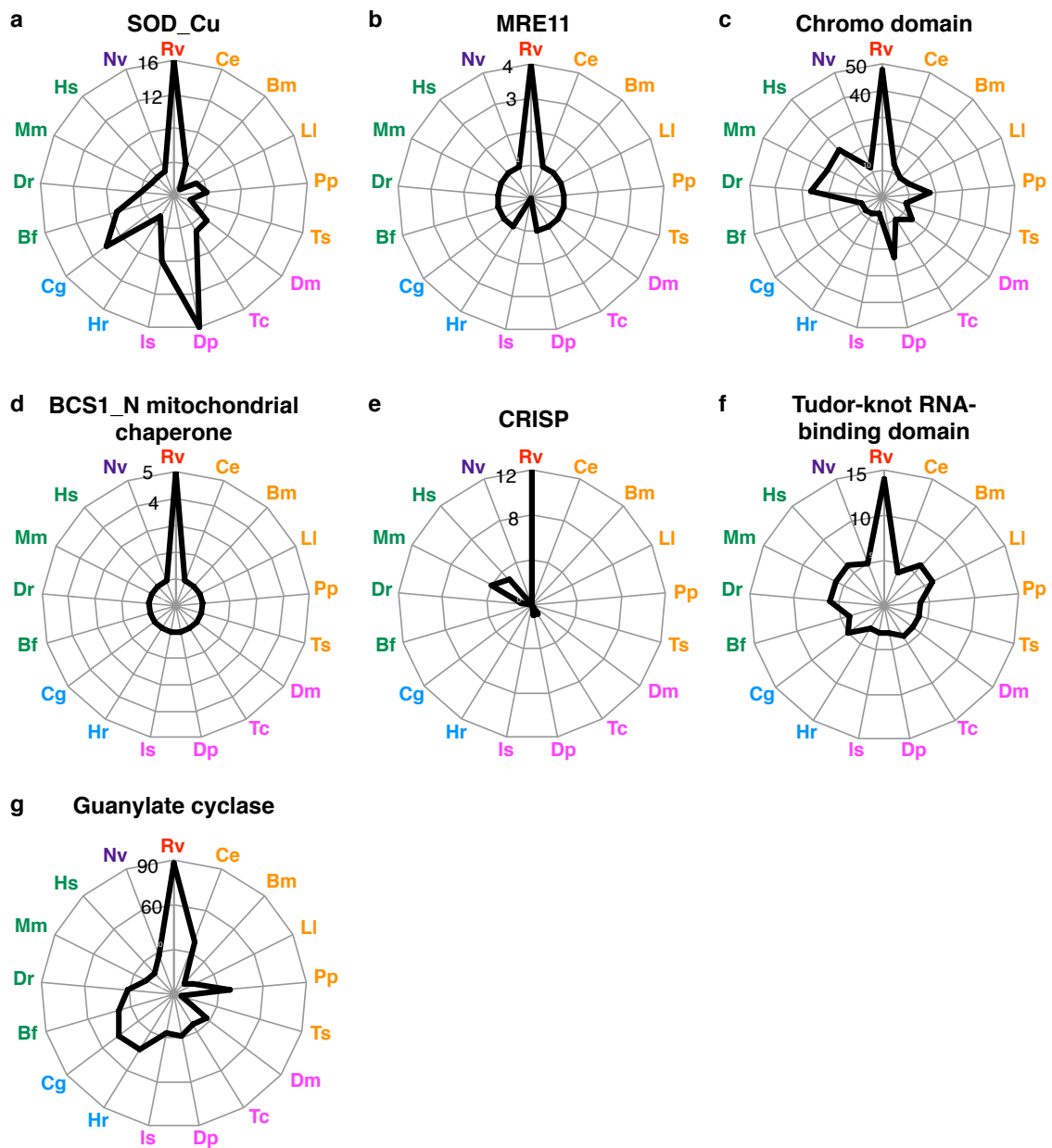
Schematic representation of metazoan catalases is shown from three model metazoans, *Homo sapiens* (Hs; NCBI gi=4557014), *Drosophila melanogaster* (Dm; Flybase name=Cat-PA, CG9314-PA), and *Caenorhabditis elegans* (Ce; Wormbase ID=CTL-1, CTL-2, CTL-3 isoform a). All of typical metazoan catalases comprised approximately 500 residues and contain Catalase domain (Pfam PF00199) and Catalase-rel domain (Pfam PF06628). All four catalases of *Ramazzottius varieornatus* (Rv) contain an extra domain (Superfamily annotation database, SSF52317; class I glutamine amidotransferase-like) at the C-terminus, and are longer than 700 residues except for a putative pseudo gene, Rv\_Catalase2. As representative bacterial clade II catalases, a schematic structure of hydroperoxidase II (HP11) of *Escherichia coli* (Escherichia; gi=115722) and *Pseudomonas fuscovaginae* (Pseudomonas; gi=518192420) are shown.





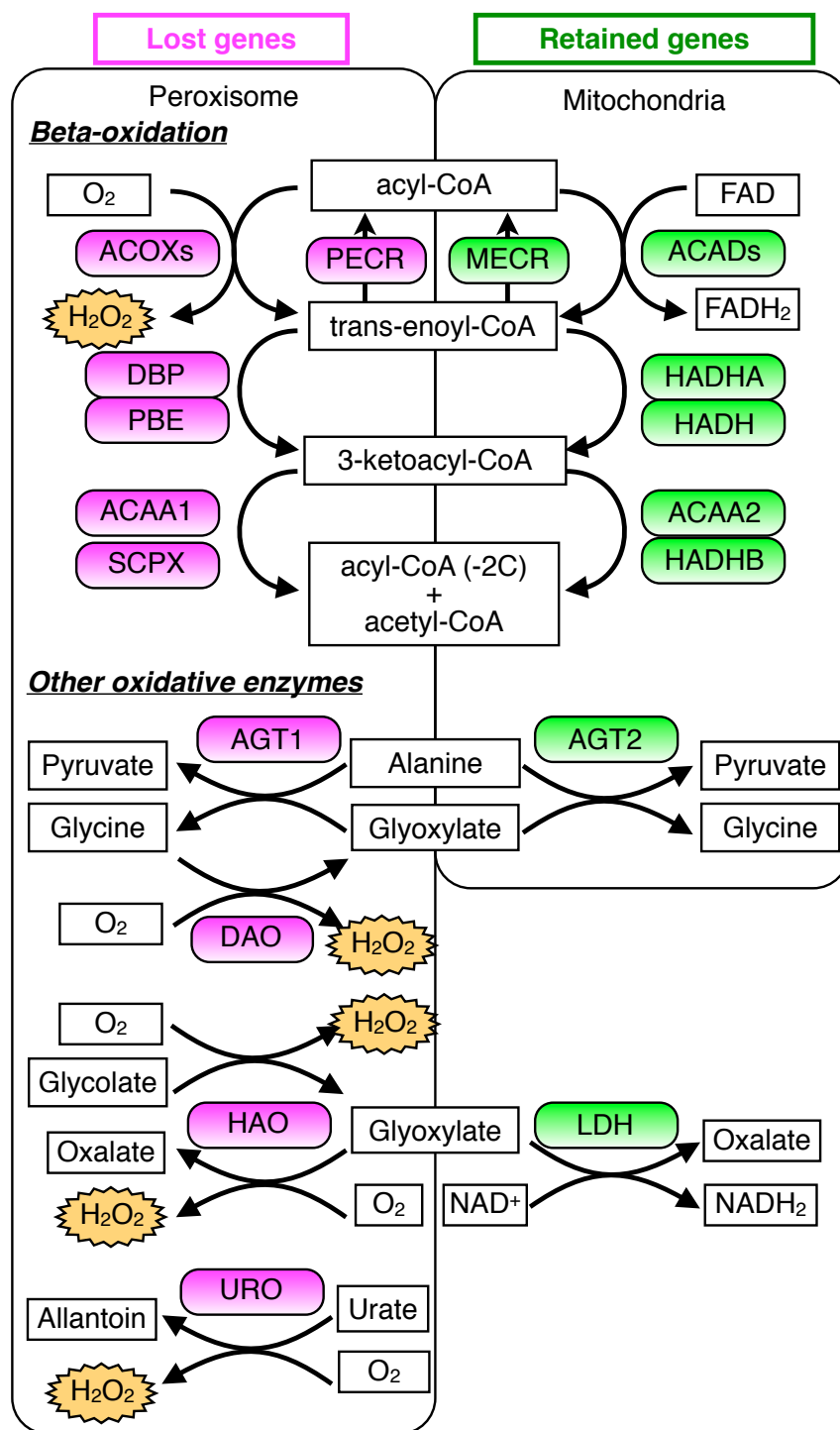
**Supplementary Figure 5. Phylogenetic analysis of catalases**

Protein sequences of the Catalase domain from various species, such as bacteria (black), plants (green), fungi (blue), metazoa (magenta), and tardigrades (red) were aligned using ClustalW and phylogenetic tree was constructed by maximum likelihood using PhyML. The bootstrap values (100 trials) are shown at the nodes. All metazoan catalases (magenta) formed a single clade belong to clade III, whereas all catalases of *R. varieornatus* formed a single clade in clade II.



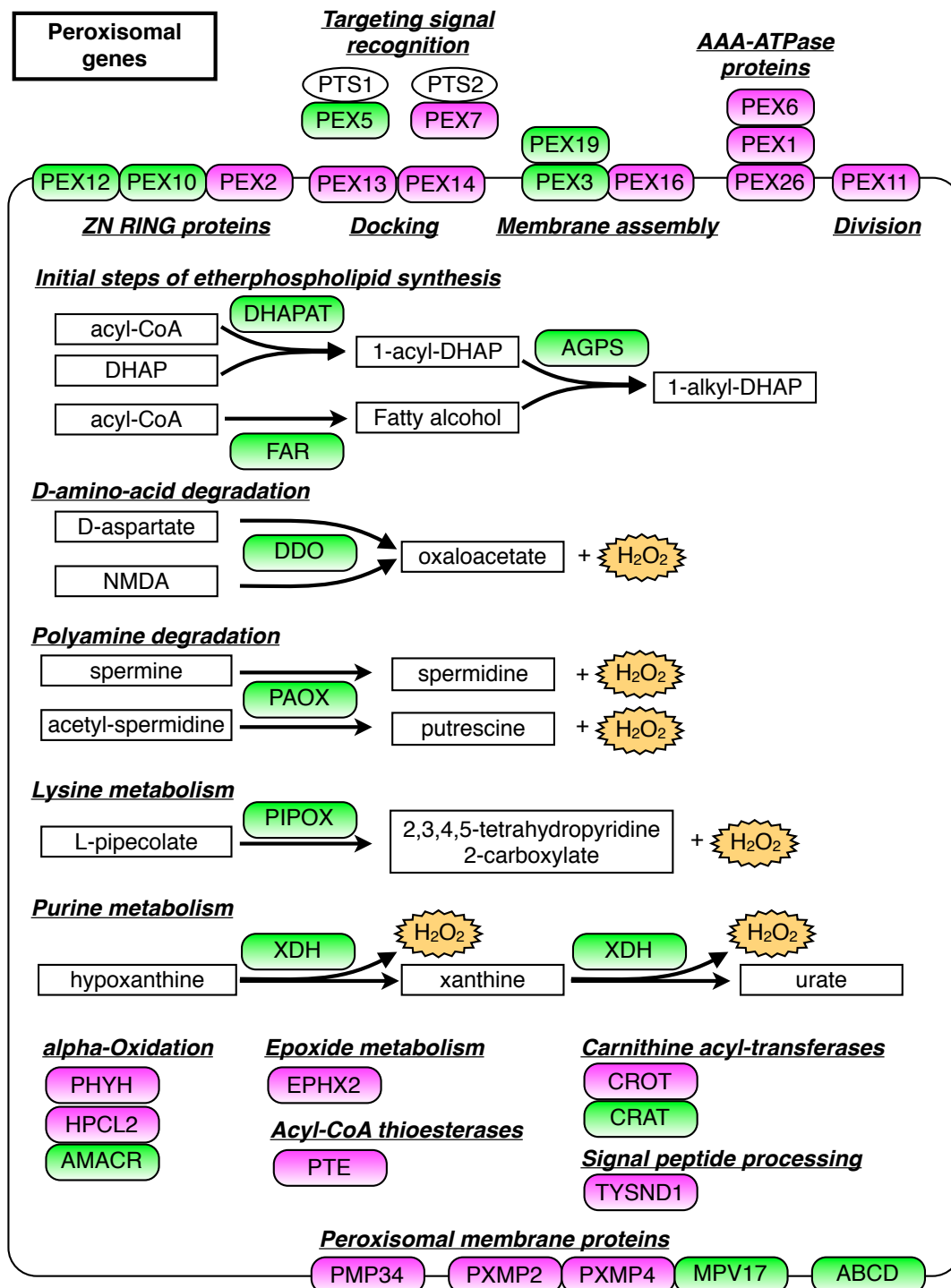
### Supplementary Figure 6. Expanded gene families in *R. varieornatus*

The gene numbers were compared among various animal species, such as tardigrade (red), nematodes (orange), arthropods (magenta), lophotrochozoa (blue), chordates (green) and cnidarian (purple). Abbreviations of species name are listed in Supplementary Data 3. Gene expansion was detected by Pfam motif search; (a) copper/zinc superoxide dismutase, (b) DNA repair enzyme, MRE11, (c) Chromo (Chromatin organization modifier) domain, (d) BCS1\_N mitochondrial chaperone, (e) CRISP (Cysteine-rich secretory protein) domain, (f) Tudor-knot RNA-binding domain, (g) guanylate cyclases.



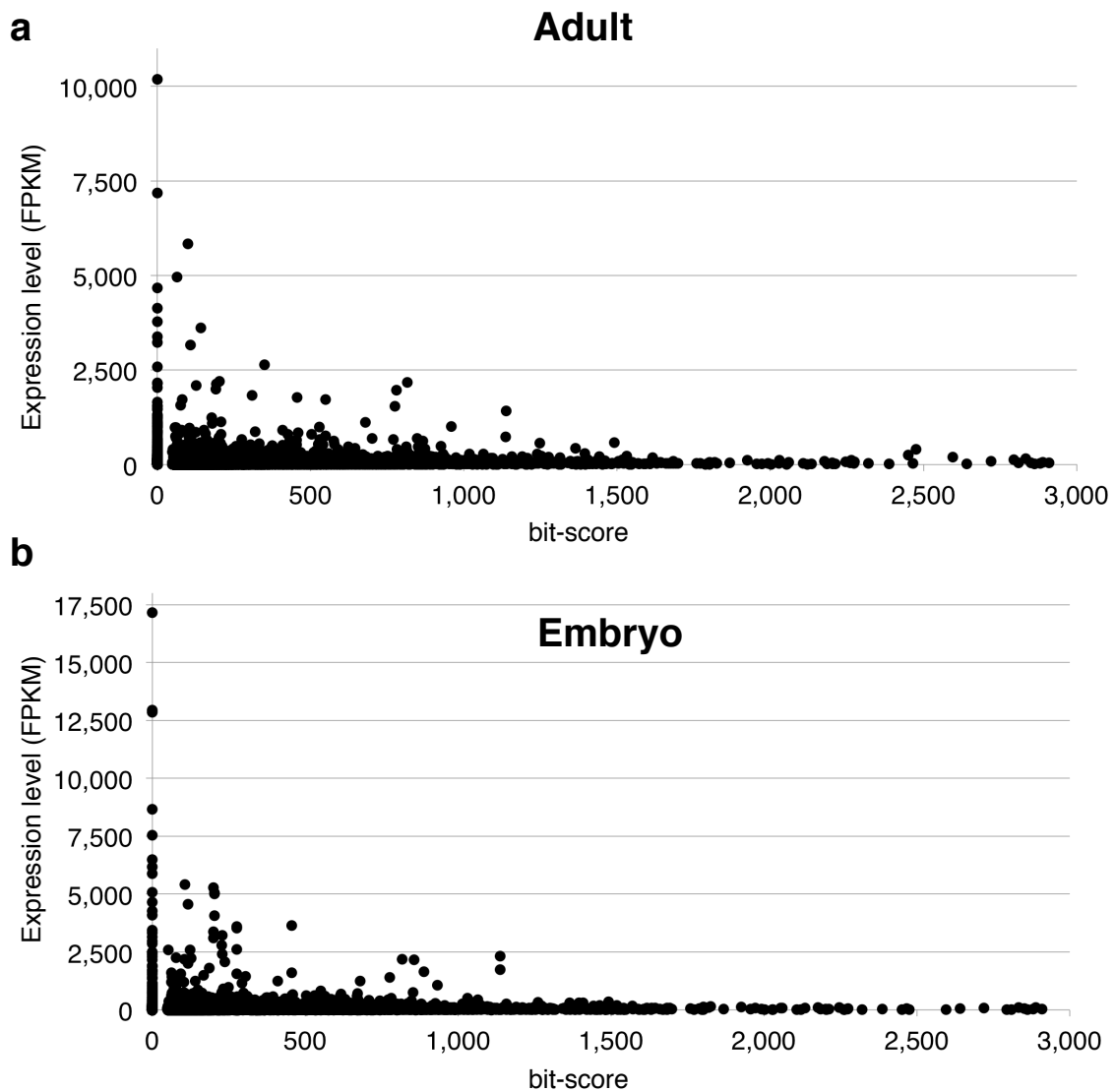
**Supplementary Figure 7. Selective loss of peroxisomal beta-oxidation pathway and other oxidative enzymes**

All genes involved in the peroxisomal beta-oxidation process were missing from our *R. varieornatus* genome, whereas all counterparts in the mitochondrial beta-oxidation process were present. Several other peroxisomal oxidative enzymes are also missing from the genome. Genes are indicated as round-squares (missing genes in magenta; retained genes in green). Metabolites other than hydrogen peroxide are shown in boxes. Gene nomenclature is according to KEGG pathway of the peroxisome (map No.04146).



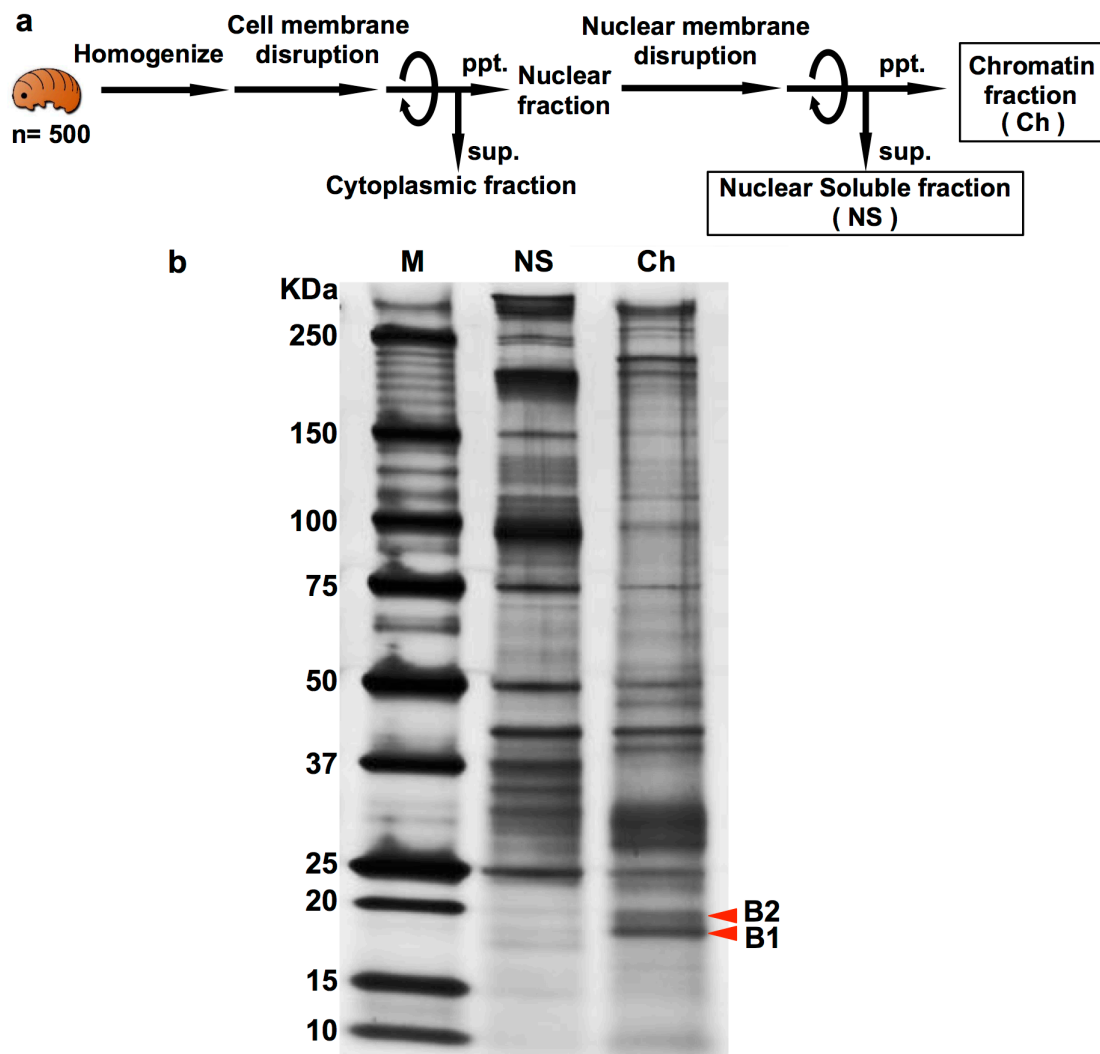
### Supplementary Figure 8. Massive loss of peroxisomal genes

Many peroxisomal genes including peroxisomal biogenesis factors (PEXs) were missing in *R. varieornatus* genome. Some genes were retained, including all genes involved in etherphospholipid synthesis, suggesting that certain peroxisomal functions are preserved in the tardigrade. Genes are indicated by round-squares (lost genes in magenta; retained genes in green) with nomenclature according to the KEGG pathway of the peroxisome (map No.04146), as similar to Supplementary Fig. 7.



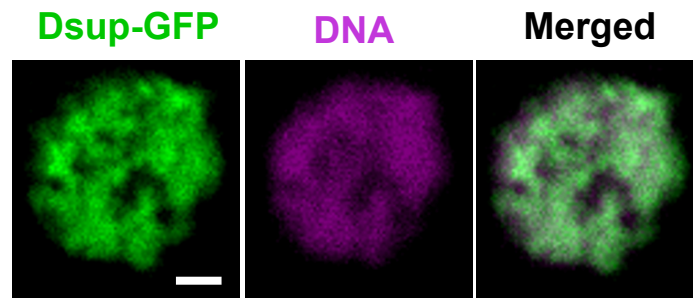
**Supplementary Figure 9. Abundant expression of genes unique to the tardigrade**

Many abundantly expressed genes in *R. varieornatus* had no or relatively low similarity to non-tardigrade genes in NCBI nr databases, in either the adult stage (a) or embryonic stage within 2 d after egg laying (b).



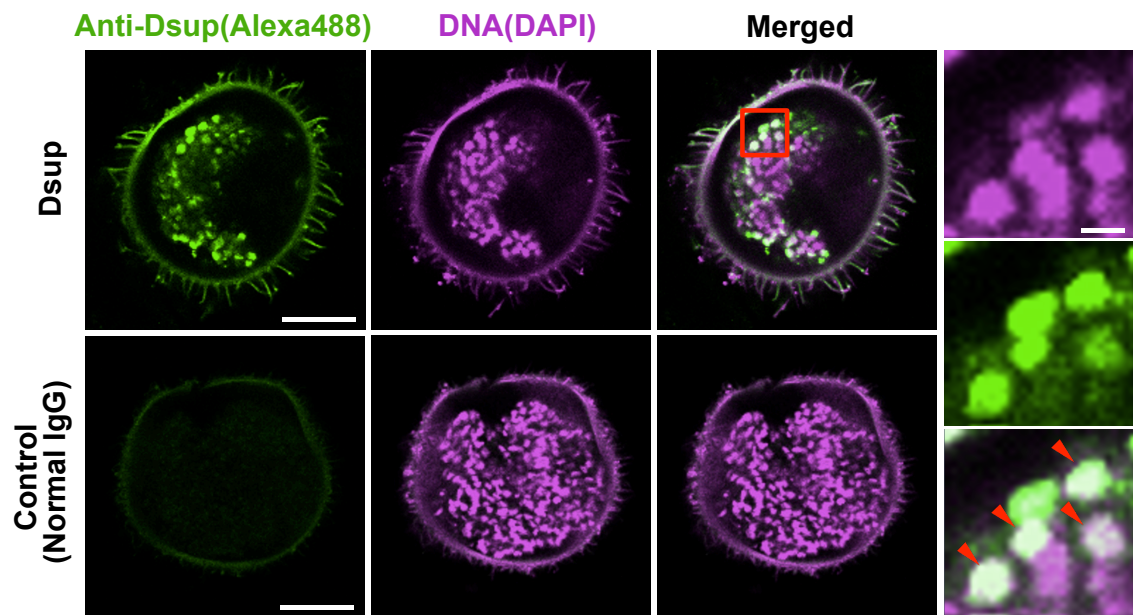
**Supplementary Figure 10. Isolation of chromatin fraction from tardigrades**

(a) Schematic procedures to isolate the chromatin fraction from *R. varieornatus*. Rotating arrows indicate centrifugation to separate supernatant (sup.) and precipitate (ppt.). (b) Silver staining of SDS-PAGE gel separating nuclear soluble fraction (NS) and chromatin fraction (Ch). Two selective bands, B1 and B2, were detected in the chromatin fraction as indicated by red arrows and were subjected to tandem mass spectrometry.



**Supplementary Figure 11. Colocalization of Dsup protein with nuclear DNA in insect cells**

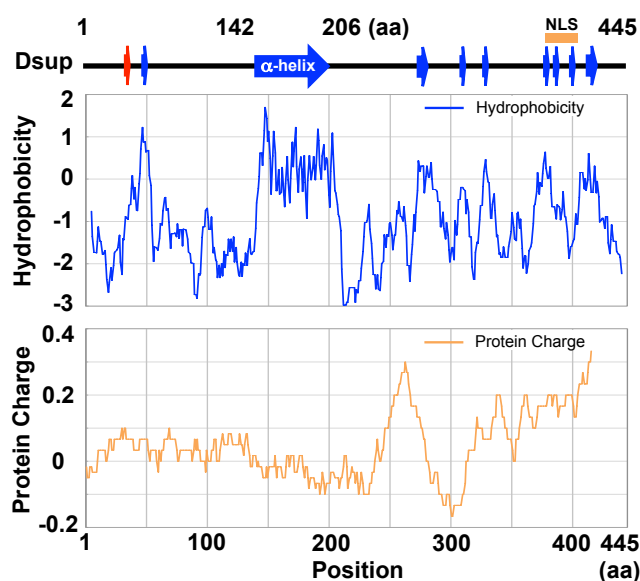
Confocal fluorescent images of *Drosophila* Schneider 2 (S2) cells expressing Dsup-GFP fusion protein. The localization of Dsup-GFP protein was merged well with the distribution of DAPI-stained nuclear DNA. The expression construct was constructed by inserting Dsup coding sequence to *Bam*HI and *Spe*I sites, and AcGFP1 to *Kpn*I and *Not*I sites of pPacPL vector. Transfection was performed using Cellfectin reagent (Invitrogen). Scale bar, 2  $\mu$ m.



**Supplementary Figure 12. Colocalization of Dsup proteins with nuclear DNA in embryonic cells of *R. varieornatus***

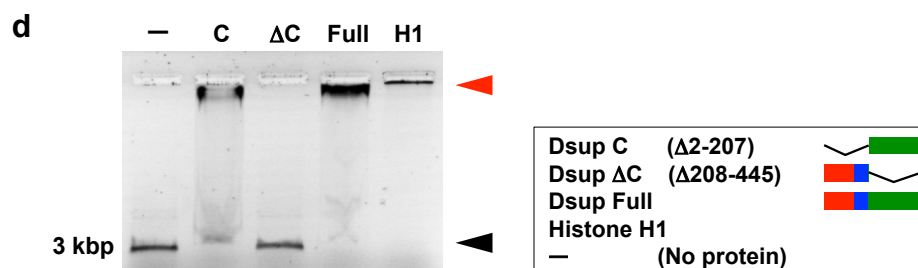
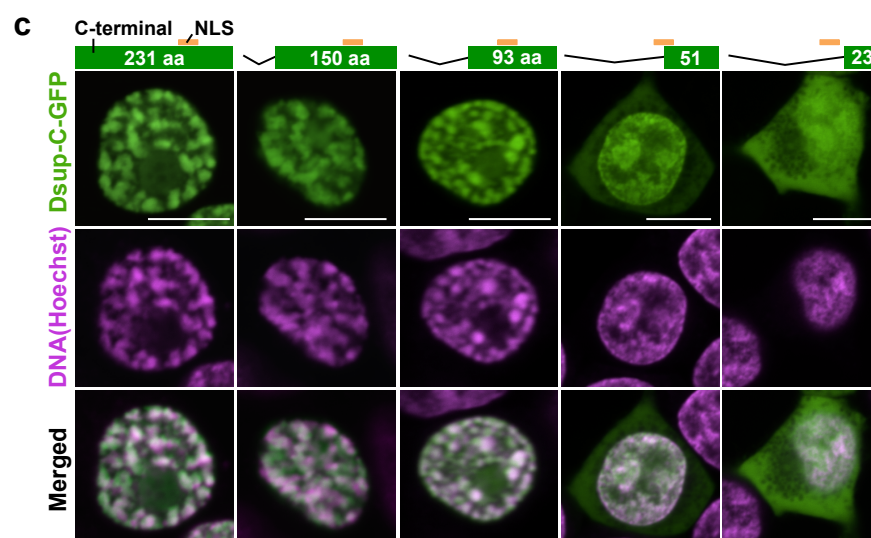
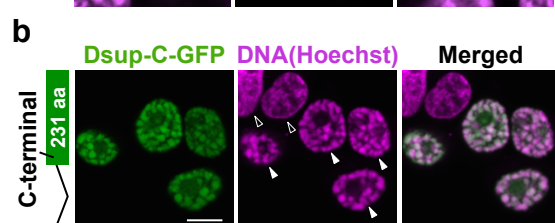
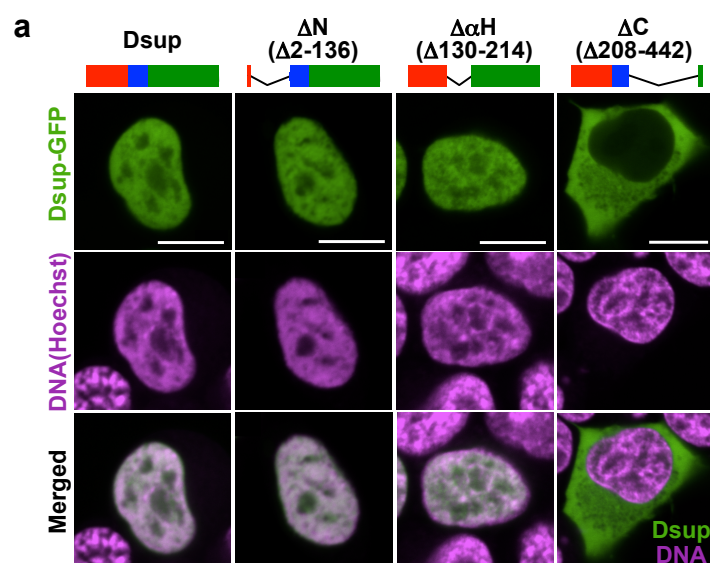
Immunohistochemical detection of Dsup proteins in a frozen section of tardigrade embryos. DNA stained with DAPI is shown in magenta. Enlarged images corresponding to the red box in the merged picture are shown on the right side. Red arrowheads indicate colocalization of Dsup proteins with nuclear DNA. Scale bar, 10  $\mu\text{m}$  (In enlarged image, scale bar indicates 2  $\mu\text{m}$ ).





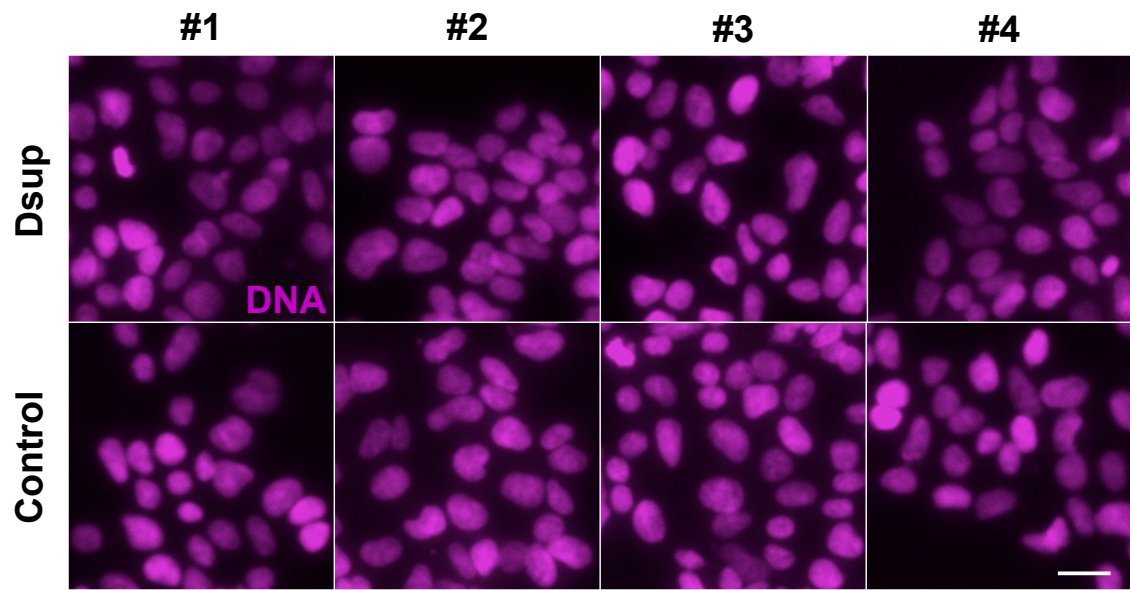
**Supplementary Figure 13. *In silico* analysis based on the Dsup protein primary structure**

Schematic representation of Dsup protein with predicted secondary structures and plots of hydrophobicity and protein charges. Blue and red arrows indicate predicted  $\alpha$ -helix and  $\beta$ -strand regions, respectively. A putative long alpha-helical region in the middle of the protein (142-206 amino-acids, aa) corresponds to a characteristically hydrophobic region in the hydropathy plot. Orange bar around the C-terminus indicates the predicted nuclear localization signal (NLS, 383-404 aa). A charge plot revealed that basic amino acids were enriched in the C-terminal region, suggesting potential involvement of the C-terminal region in the nuclear localization and DNA-association.



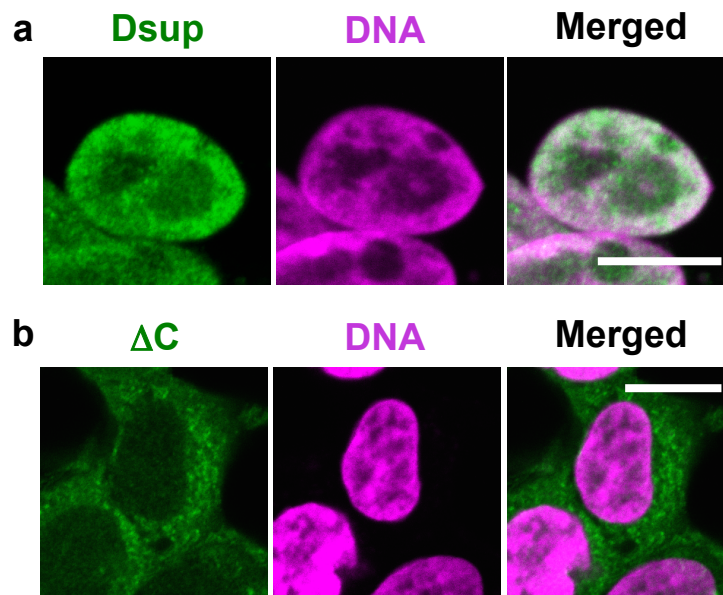
**Supplementary Figure 14. C-Terminal region is responsible for colocalization with nuclear DNA and mobility shift of DNA *in vitro***

(a) Subcellular localization of Dsup-GFP fusion proteins with deletion of the N-terminus ( $\Delta N$ ,  $\Delta 2$ -136 aa),  $\alpha$ -helical region ( $\Delta \alpha H$ ,  $\Delta 130$ -214 aa), and C-terminus ( $\Delta C$ ,  $\Delta 208$ -442 aa) in transiently transfected HEK293 cells. Schematic structures are shown above the images. Scale bars, 10  $\mu m$ . (b) C-terminal region of Dsup protein (Dsup-C) alone is sufficient for colocalization with nuclear DNA. Closed arrowheads indicate nuclei of Dsup-C-GFP expressing cells and open arrowheads indicate nuclei of non-expressing cells. Dsup-C-GFP expressing cells exhibited aggregated distributions of nuclear DNA. Scale bars, 10  $\mu m$ . (c) Subcellular localization of Dsup-GFP fusion proteins with Dsup-C deletion derivatives. Schematic structures are shown above the images with the number of remaining amino acids (aa). Scale bars, 10  $\mu m$ . (d) Effect of Dsup protein lacking the C-terminal region on DNA mobility. Black arrowhead indicates the predicted size of the unbound linear probe DNA (3 kbp, 10 ng). Red arrowhead indicates the position of the extremely slowly migrating DNA in the presence of full-length Dsup protein (Full, 100 ng) or C-terminal region alone (C,  $\Delta 2$ -207 aa, 100 ng). The C-terminal region alone was sufficient to shift DNA mobility similar to that of full-length Dsup protein. In contrast, Dsup protein lacking the C-terminal region ( $\Delta C$ ,  $\Delta 208$ -445 aa, 100 ng) completely lost the ability to shift the DNA mobility, suggesting that the C-terminal region is essential for the association with DNA. Histone H1 (H1, 100 ng) was used as a control.



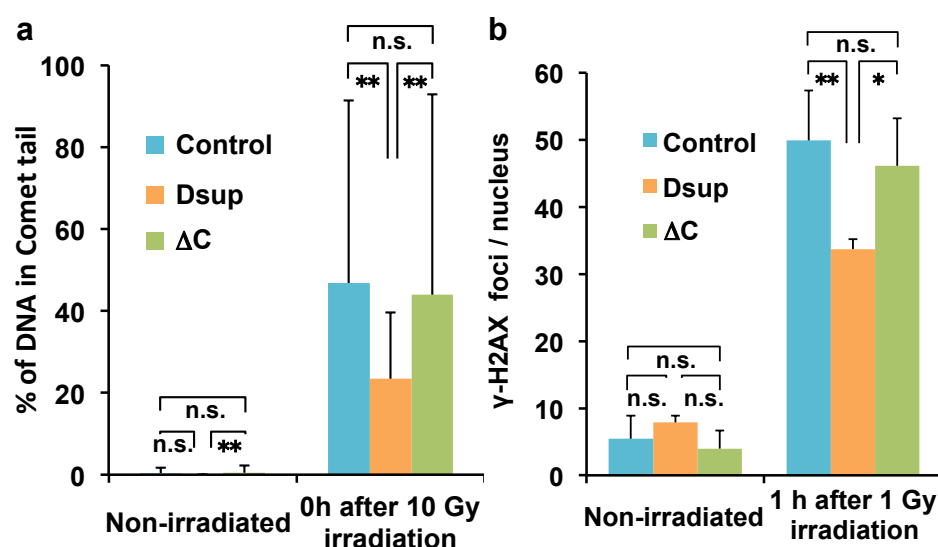
**Supplementary Figure 15. Comparison of the DAPI-stained images between Dsup-expressing cells and control cells**

The stable line expressing Dsup (Dsup) and control HEK293 cells (Control) were stained with DAPI after fixation with 4% formaldehyde. No significant difference was observed in distribution of nuclear DNA and fluorescence strength between Dsup-expressing cells and control cells. The fluorescent images were captured using a fluorescent microscope (BZ-9000, Keyence). Scale bar, 20  $\mu\text{m}$ .



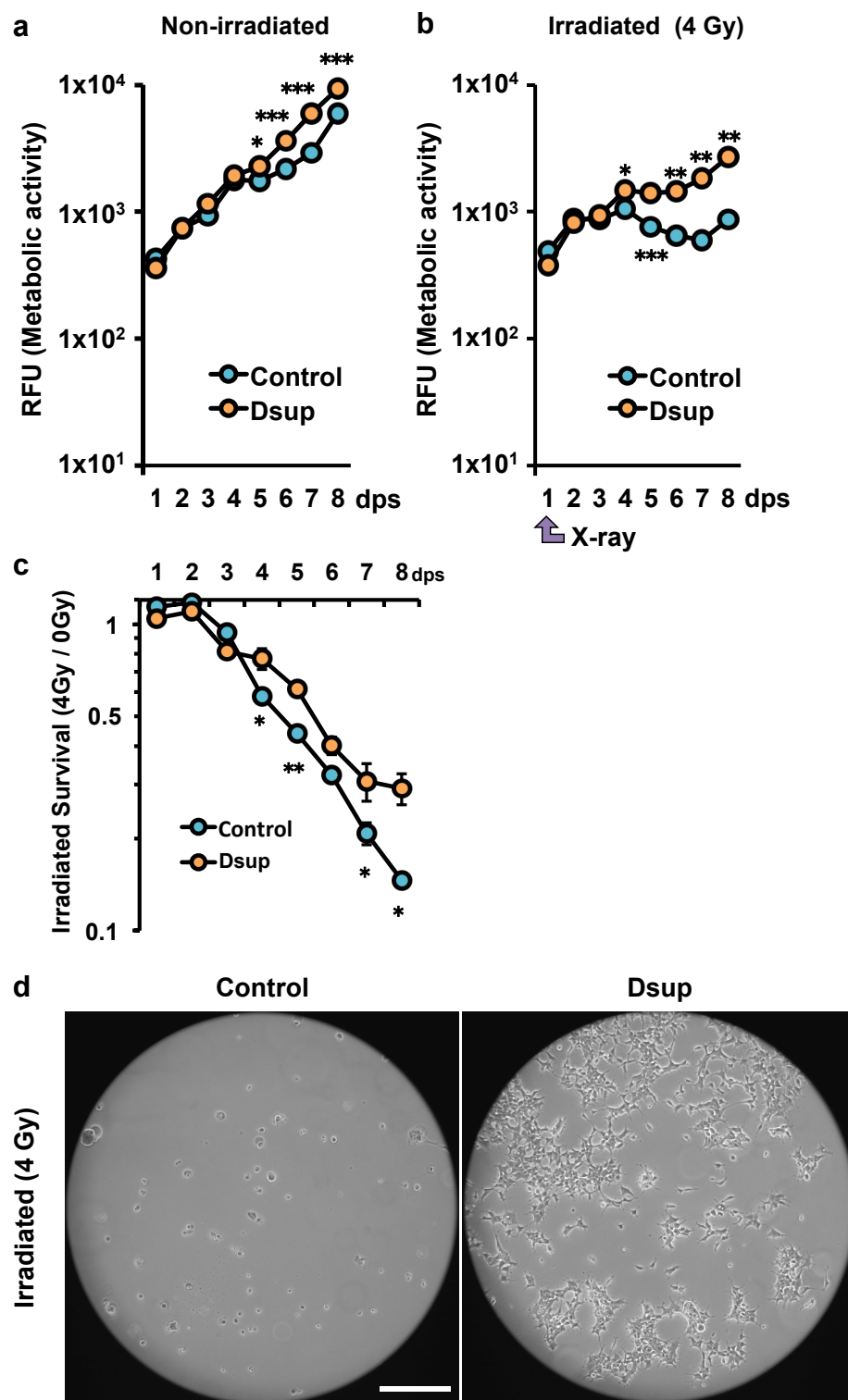
**Supplementary Figure 16. Subcellular localization of Dsup proteins in stably transfected HEK293 cells**

Subcellular localization of Dsup proteins were examined by immunocytochemistry in stably transfected HEK293 cells expressing (a) full length Dsup (Dsup) and (b) Dsup lacking C-terminal DNA-associated region ( $\Delta C$ ). Nuclear DNA was visualized by DAPI staining. Full length Dsup protein colocalized with nuclear DNA, while Dsup $\Delta C$  localized mainly in the cytoplasm. Scale bars, 10  $\mu\text{m}$ .



### Supplementary Figure 17. Requirement of the C-terminal region of Dsup protein for DNA protection activity in Dsup-expressing cells

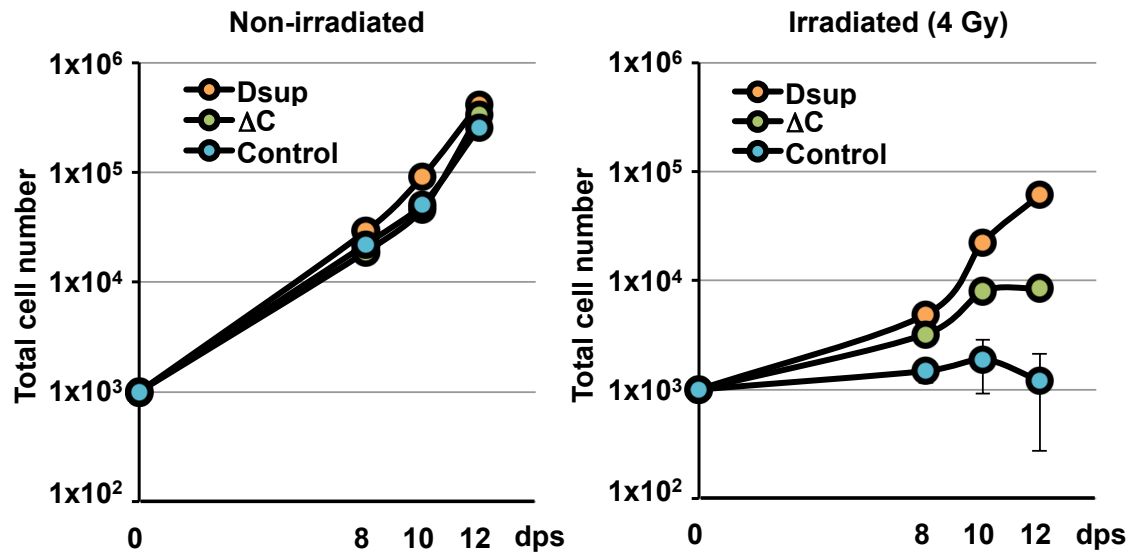
The effects of full-length Dsup and Dsup lacking the C-terminal region (DsupΔC) on DNA breaks caused by X-ray were analysed using (a) an alkaline comet assay and (b) a  $\gamma$ -H2AX assay. (a) In the alkaline comet assays, DNA fragmentation was assessed as the proportion of DNA detected in the tail region (% of DNA in Comet tail) and compared among untransfected HEK293 cells (Control), full-length Dsup-expressing cells (Dsup) and DsupΔC-expressing cells (ΔC). In contrast to Dsup-expressing cells, which exhibited approximately 50% reduction in the proportion of fragmented DNA, DsupΔC-expressing cells exhibited no reduction compared to control cells. At least 120 comets were analysed for each condition. (b) Quantitative comparison of  $\gamma$ -H2AX foci number under non-irradiated and X-ray irradiated conditions. At least 62 nuclei were analysed for each condition. Values represent mean  $\pm$  s.d.. \* $P$ <0.05, \*\* $P$ <0.01, n.s. indicates not significant (Tukey-Kramer's test).



Supplementary Figure 18. Effect of Dsup on cell viability of human cultured cells under irradiated and non-irradiated conditions

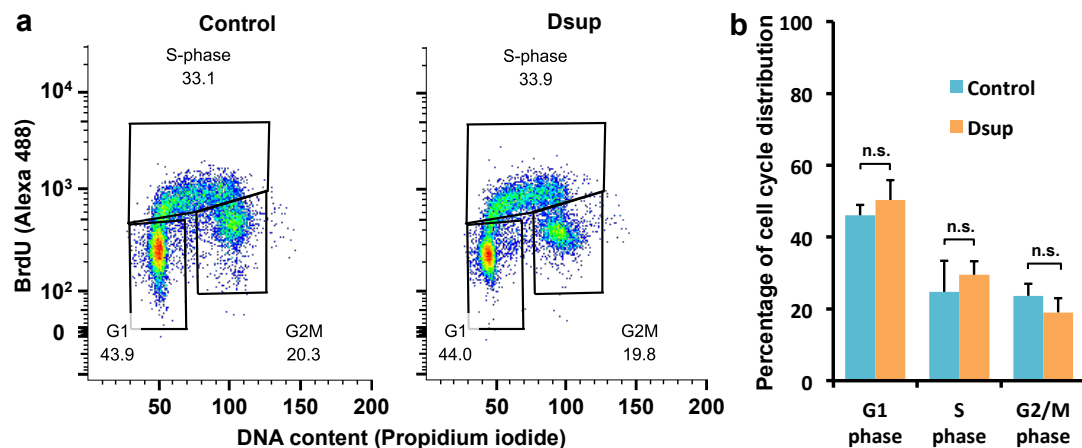
(**a, b**) Comparison of temporal changes in cell viability between untransfected HEK293 cells (Control) and Dsup-expressing cells (Dsup) under non-irradiated (**a**) and irradiated conditions with 4 Gy of X-ray (**b**). Cellular viability was measured using PrestoBlue reagent detecting reducing power of total cells. RFU, relative fluorescence units. (**c**) Temporal change of irradiated survival, which is cell viability in irradiated condition normalized with that in the non-irradiated condition at the same time point. (**d**) Phase contrast microscopy images of 4 Gy X-ray irradiated cells at 12 dps. dps, days post seeding. Scale bar, 200  $\mu$ m. Values represent mean  $\pm$  s.e.m.. \* $P$ <0.05, \*\* $P$ <0.01, \*\*\* $P$ <0.001 (Student's  $t$ -test).





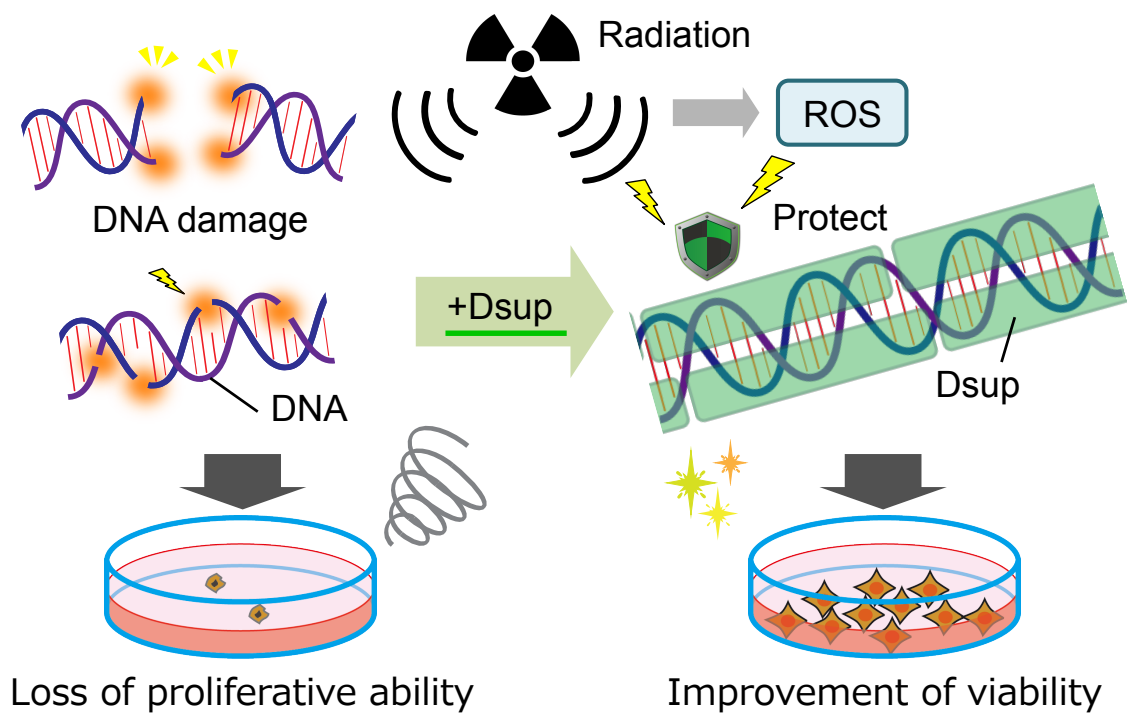
**Supplementary Figure 19. Effect of C-terminal region of Dsup protein on improved cell proliferative ability in irradiated Dsup-expressing cells**

Comparison of the cell proliferation curves of parental HEK293 cells (Control), Dsup-expressing cells (Dsup), and DsupΔC-expressing cells (ΔC) under non-irradiated and irradiated conditions. Values represent mean  $\pm$  s.d.. dps, days post seeding.



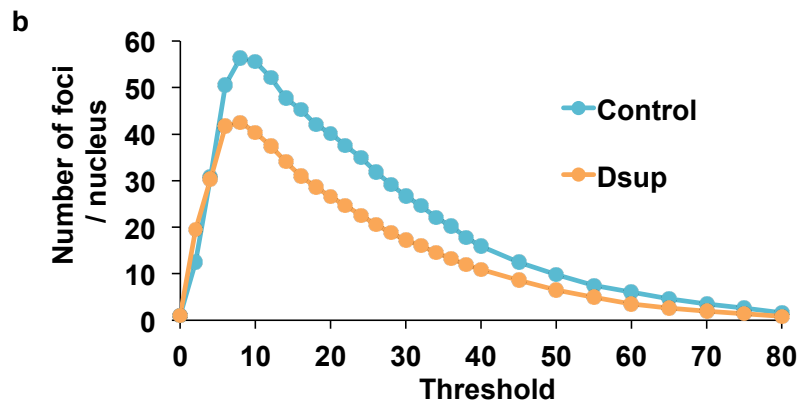
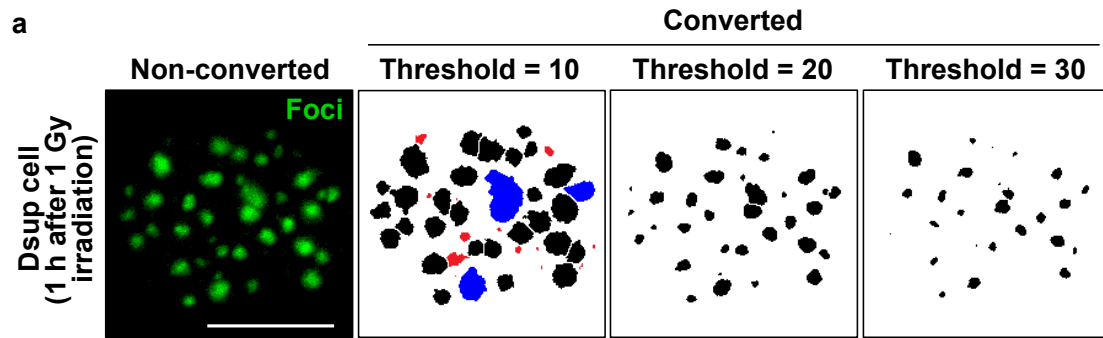
### Supplemental Figure 20. Cell cycle distribution of Dsup-expressing cells

(a) Representative dot plots of cell cycle distribution of HEK293 cells (Control) and Dsup-expressing cells (Dsup). Cell cycle was analysed by combined propidium iodide and BrdU staining. At least 10,000 events were collected and the distribution of cell cycle phases (G1 phase, S phase and G2/M phase) was calculated using gated regions. (b) Quantitative comparison of cell cycle between HEK293 cells (Control) and Dsup-expressing cells (Dsup) for each phase. The statistical analysis of the three experiments is shown. Values represent mean  $\pm$  s.d.. n.s. indicates not significant (Welch's *t*-test. G1 phase; *t*-value = -1.190, *p*-value = 0.320. S phase; *t*-value = -0.872, *p*-value = 0.448. G2/M phase; *t*-value = 1.548, *p*-value = 0.197).



**Supplementary Figure 21. Schematic model of DNA protection by Dsup protein from radiation damage**

Radiation induces DNA damage, such as double-strand breaks (DSBs) and single-strand breaks (SSBs), which interfere DNA replication and gene expression. Heavily damaged cells lose their proliferative ability and are destined to death. Dsup protein associated with nuclear DNA could be beneficial to suppress X-ray induced DNA damage through physical shielding or protection from indirect radiation effects (reactive oxygen species, ROS). Thereby, Dsup protein could improve the radiotolerance of cultured animal cells.



**c**

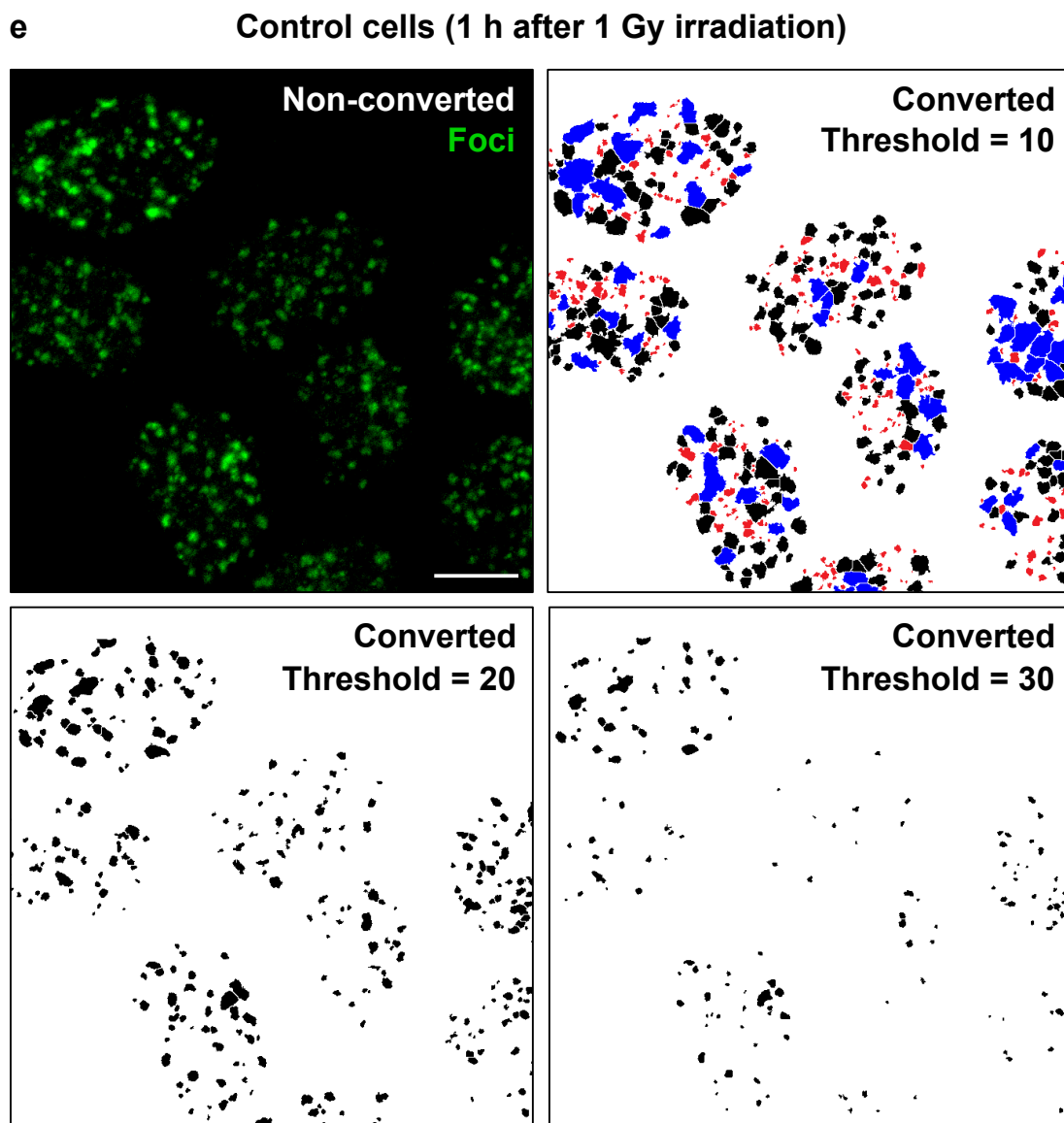
| Cell line | Threshold value | Total number of foci | Foci size       |        |                 | The number of nucleus | The number of foci / nucleus |
|-----------|-----------------|----------------------|-----------------|--------|-----------------|-----------------------|------------------------------|
|           |                 |                      | 25th percentile | median | 75th percentile |                       |                              |
| Control   | 20              | 2809                 | 33              | 73     | 144             | 70                    | 40.13                        |
| Dsup      | 20              | 2443                 | 31              | 68     | 132             | 92                    | 26.55                        |
|           | 18              | 2635                 | 32              | 72     | 140.5           | 92                    | 28.64                        |
|           | 17              | 2773                 | 33              | 73     | 147             | 92                    | 30.14                        |
|           | 16              | 2852                 | 34              | 76     | 155             | 92                    | 31.00                        |

**d**

| Comparison pair (Threshold) | $p$ -value (F-test) <sup>a</sup> | Statistical method | $t$ -value | $p$ -value | Significance <sup>b</sup> |
|-----------------------------|----------------------------------|--------------------|------------|------------|---------------------------|
| Control (20) vs. Dsup (20)  | 8.2E-02                          | Welch's $t$ -test  | 5.869      | 1.9E-08    | ***                       |
| Control (20) vs. Dsup (17)  | 4.8E-02                          | Welch's $t$ -test  | 4.169      | 2.8E-05    | ***                       |

<sup>a</sup> The equality of variances was tested using F-test (the significance level = 0.05).

<sup>b</sup> \*\*\* $P < 0.001$



**Supplementary Figure 22. The effect of the image conversion threshold values on visual output and the detection of  $\gamma$ -H2AX foci**

The numbers of  $\gamma$ -H2AX foci were counted after conversion of the original fluorescent image to a binarized image. The threshold value for image conversion was manually adjusted, until a visually best fit between the original (non-converted) and converted images of irradiated Dsup cells were observed for each experiment. (a) Representative non-converted fluorescent image and the converted images using the various threshold values of irradiated

Dsup-expressing cells. The image converted with the threshold value of 20 produced the best fit to the original fluorescent image. Application of the lower threshold value (10) produced artificial fusions of neighboring foci (shown in blue) and erroneous detections of small dim (background-level) fluorescent signals (shown in red). The threshold value of 30 reduced the foci signals too much. Similar results were observed in irradiated control HEK293 cells (**e**). Scale bars, 10 $\mu$ m. (**b**) The effect of the threshold values on the mean number of detected  $\gamma$ -H2AX foci per nucleus. Under the irradiated condition, Dsup-expressing cells (Dsup) exhibited a constantly lower number of foci than that in untransfected HEK293 cells (Control) except the occasional case with the threshold value of 2. (**c**) Quantitative comparison of the foci sizes between control cells and Dsup-expressing cells with various conversion thresholds. With the threshold of 20, the foci size of Dsup-expressing cells is smaller than that of control cells. With the threshold value of 17, enhancing the detection sensitivity, Dsup cells exhibited a distribution of foci sizes similar to that of control cells converted with the threshold of 20. (**d**) Pairwise statistical analysis of the foci number between Dsup-expressing cells and control cells under irradiated condition. Regardless of whether the same threshold value (20) was used for two cell lines, or more sensitive threshold value (17) was used for Dsup-expressing cells to equalize the foci size distribution between two cell lines, Dsup-expressing cells exhibited statistically significant decrease in foci number compared to that of control cells (converted with the threshold of 20), robustly confirming that Dsup suppressed DNA breaks.

## Supplementary Tables

**Supplementary Table 1. Summary of sequencing reads**

| Sequencing                         | Platform            | # of reads  | Total bases    | Valid bases    |
|------------------------------------|---------------------|-------------|----------------|----------------|
| <b>WGS</b>                         | ABI3730xl           | 772,854     | 951,424,325    | 925,851,199    |
| <b>GSS</b>                         | ABI3730xl           | 42,446      | 55,303,419     | 52,412,436     |
| <b>EST</b>                         | ABI3730xl           | 70,819      | 85,333,501     | 80,585,982     |
| <b>Illumina<br/>genomic reads</b>  | GAllx - 75PE        | 119,748,430 | 8,981,132,250  | 8,964,079,477  |
| <b>mRNA-seq<br/>adult - active</b> | GAllx - 75PE        | 39,770,026  | 2,982,751,950  | 2,977,058,737  |
| <b>mRNA-seq<br/>adult - tun</b>    | GAllx - 75PE        | 39,162,810  | 2,937,210,750  | 2,931,728,395  |
| <b>mRNA-seq<br/>adult - reh80m</b> | GAllx - 75PE        | 38,650,446  | 2,898,783,450  | 2,892,887,719  |
| <b>mRNA-seq<br/>adult - reh3h</b>  | GAllx - 75PE        | 37,177,272  | 2,788,295,400  | 2,782,904,822  |
| <b>mRNA-seq<br/>embryo2d</b>       | Hiseq2000 -<br>76PE | 129,195,500 | 9,818,858,000  | 9,802,835,817  |
| <b>mRNA-seq<br/>embryo4d</b>       | Hiseq2000 -<br>76PE | 144,845,374 | 11,008,248,424 | 10,985,546,847 |

**Supplementary Table 2.**  
**Statistics of genome assembly and gene models of *R. varieornatus***

| Scaffolds                  | Unfiltered assembly<br>(≥1kbp) | Final Assembly<br>with high coverage |
|----------------------------|--------------------------------|--------------------------------------|
| Number of scaffolds        | 301                            | 199                                  |
| Span (bp)                  | 55,981,880                     | 55,828,384                           |
| Valid bases without N (bp) | 55,552,630                     | 55,409,081                           |
| GC (%)                     | 47.5%                          | 47.5%                                |
| Longest length (bp)        | 9,333,084                      | 9,333,084                            |
| Shortest length (bp)       | 1,000                          | 1,000                                |
| Average length (bp)        | 185,986                        | 280,544                              |
| N50 length (bp)            | 4,740,345 (#4)                 | 4,740,345 (#4)                       |
| N90 length (bp)            | 1,295,620 (#15)                | 1,295,620 (#15)                      |
| Mapped EST (%) *           | 99.80%                         | 99.80%                               |
| Mapped GSS (%) **          | 99.71%                         | 99.70%                               |
| Contigs                    | Unfiltered assembly            | Final assembly                       |
| Number of contigs          | 1,077                          | 963                                  |
| Longest length (bp)        | 808,962                        | 808,962                              |
| Shortest length (bp)       | 641                            | 695                                  |
| Average length (bp)        | 51,580                         | 57,538                               |
| N50 length (bp)            | 127,399 (#123)                 | 127,441 (#122)                       |
| N90 length (bp)            | 34,856 (#443)                  | 36,155 (#439)                        |
| CEGMA completeness         | Unfiltered assembly            | Final assembly                       |
| Complete (%)               | 95.56%                         | 95.56%                               |
| Average copy (in complete) | 1.13                           | 1.13                                 |
| Partial (%)                | 96.77%                         | 96.77%                               |
| Average copy (in partial)  | 1.20                           | 1.19                                 |
| Gene model                 | Unfiltered assembly            | Final assembly                       |
| Number of genes            | 19,673                         | 19,521                               |
| Putative HGT genes         | 363                            | 234                                  |
| Proportion of HGT (%)      | 1.8%                           | 1.2%                                 |

\* Proportion of 70,819 EST sequences successfully mapped using BLASTN (e-value  $\leq 10^{-65}$ ).

\*\* Proportion of 42,446 GSS sequences successfully mapped using BLASTN (e-value  $\leq 10^{-65}$ ).



**Supplementary Table 3.**  
**Summary of the final gene model of *R. varieornatus***

|                         | Average | S.D.  |
|-------------------------|---------|-------|
| Number of genes         | 19,521  |       |
| CDS length (bp)         | 1,062   | 1,178 |
| Number of exons / gene  | 4.5     | 4.6   |
| Exon length (bp)        | 234     | 210   |
| Intron length (bp)      | 402     | 890   |
| Inter-CDS distance (bp) | 1,099   | 1,370 |

**Supplementary Table 4. Identified proteins from the chromatin fraction selective bands**

| Band | ID               | Mass (Da) | PSORT <sup>a</sup>  | TargetP <sup>b</sup> | BLASTP/Pfam                          |
|------|------------------|-----------|---|----------------------|--------------------------------------|
| B1   | RvY_06764        | 21,332    | extr: 30  | SP: 0.861            | Chitin binding Peritrophin-A domain  |
|      | RvY_16129        | 98,902    | extr: 20, ER: 4, lyso: 4, pero: 2                             | SP: 0.973            | N.A.                                 |
|      | RvY_17224 (Dsup) | 42,823    | nucl: 20.5, cyto_nucl: 14.5, cyto: 7.5                        | other: 0.910         | N.A.                                 |
|      | RvY_04937        | 70,157    | plas: 11, ER: 9.5, ER_golg: 6, extr: 4, mito: 2, pero: 2      | other: 0.575         | N.A.                                 |
|      | RvY_12593        | 34,623    | nucl: 20.5, cyto_nucl: 13.5, cyto: 5.5, extr: 4               | other: 0.870         | N.A.                                 |
|      | RvY_04156        | 18,792    | mito: 21, cyto: 9   | other: 0.706         | Myosin regulatory light chain 12A    |
|      | RvY_06734        | 22,282    | extr: 30  | SP: 0.895            | Chitin binding Peritrophin-A domain  |
|      | RvY_18007        | 19,686    | extr: 31  | SP: 0.845            | Chitin binding Peritrophin-A domain  |
|      | RvY_06625        | 22,628    | plas: 28, ER: 3   | other: 0.604         | [MARVEL] Membrane-associating domain |
|      | RvY_14932        | 25,555    | plas: 28, mito: 2   | mTP: 0.522           | [MARVEL] Membrane-associating domain |
|      | RvY_00840        | 23,990    | extr: 31  | SP: 0.876            | Chitin binding Peritrophin-A domain  |
|      | RvY_06311        | 21,580    | extr: 22.5, extr_plas: 14.5, plas: 5.5                        | SP: 0.741            | N.A.                                 |
| B2   | RvY_14932        | 26,240    | plas: 28, E.R.: 3   | other: 0.476         | [MARVEL] Membrane-associating domain |
|      | RvY_06764        | 21,332    | extr: 30  | SP: 0.861            | Chitin binding Peritrophin-A domain  |
|      | RvY_18007        | 19,686    | extr: 31  | SP: 0.845            | Chitin binding Peritrophin-A domain  |
|      | RvY_04937        | 70,157    | plas: 11, E.R.: 9.5, E.R._golgi: 6, extr: 4, mito: 2, pero: 2 | other: 0.575         | N.A.                                 |
|      | RvY_06734        | 22,282    | extr: 30  | SP: 0.895            | Chitin binding Peritrophin-A domain  |
|      | RvY_18401        | 19,174    | mito: 24, nucl: 4, cyto: 2                                    | mTP: 0.552           | N.A.                                 |
|      | RvY_00840        | 23,990    | extr: 31  | SP: 0.876            | Chitin binding Peritrophin-A domain  |
|      | RvY_12594        | 31,432    | nucl: 21, cyto_nucl: 14.5, cyto: 6, extr: 4                   | other: 0.933         | N.A.                                 |
|      | RvY_15001        | 22,046    | extr: 30  | SP: 0.873            | Chitin binding Peritrophin-A domain  |
|      | RvY_04156        | 18,792    | mito: 21, cyto: 9   | other: 0.706         | Myosin regulatory light chain 12A    |
|      | RvY_04440        | 30,736    | extr: 29  | SP: 0.862            | Astacin (Peptidase family M12A)      |
|      | RvY_06311        | 21,580    | extr: 22.5, extr_plas: 14.5, plas: 5.5                        | SP: 0.741            | N.A.                                 |

<sup>a</sup> Predicted subcellular localizations are indicated by abbreviated four-letter codes. Precise descriptions of the location codes are provided in WoLF PSORT server ([http://wolfpsort.org/aboutWoLF\\_PSORT.html.en](http://wolfpsort.org/aboutWoLF_PSORT.html.en)). Proteins predicted to nucleus were highlighted in red.

<sup>b</sup> Subcellular localizations are predicted by the presence of an N-terminal signal peptide. Predicted scores are shown for mitochondrial targeting peptide (mTP), secretory signal peptide (SP), and other location (other).

**Supplementary Table 5. List of peptide detections for the identified proteins**

| Protein ID | Mascot score | Peptide sequence                   |
|------------|--------------|------------------------------------|
| RvY_06764  | 48           | VFSALDVLR                          |
|            | 65           | SPLQTDEIR                          |
|            | 54           | AATGAVLSSADSR                      |
|            | 39           | LLDDENDYELK                        |
| RvY_16129  | 59           | VTGSSQGAINQQQAK                    |
|            | 84           | VTGSSAQQIDINQAR                    |
| RvY_17224  | 84           | LTSSGTGAGSAPAAAK                   |
| RvY_04937  | 81           | NIPVGGVNTEATGDNYIR                 |
| RvY_12593  | 81           | QGGMGMSGGMGGADR                    |
| RvY_04156  | 56           | GPEDEAGGPPK                        |
|            | 48           | LTDDELNQAMK + Oxidation (M)        |
| RvY_06734  | 44           | AATGAVQSSASK                       |
|            | 48           | VLLDNQDDYELK                       |
| RvY_18007  | 48           | VFSALDVLR                          |
|            | 40           | EIYANAQPGK                         |
| RvY_06625  | 40           | QIMDNEGLR                          |
|            | 45           | QIMDNEGLR + Oxidation (M)          |
| RvY_14932  | 54           | SAPLSASPISAR                       |
| RvY_00840  | 46           | SSSSSSQESQSSSSQVR                  |
| RvY_06311  | 37           | STVGQTPPQNLR                       |
| RvY_14932  | 113          | APVNGIVTDANGNQIQVR                 |
|            | 73           | TNAAGQLIGPGGIAINDAGLTR             |
| RvY_06764  | 61           | VFSALDVLR                          |
|            | 43           | SPLQTDEIR                          |
|            | 44           | QFQDYSNSR                          |
|            | 39           | AATGAVLSSADSR                      |
| RvY_18007  | 61           | VFSALDVLR                          |
|            | 49           | EIYANAQPGK                         |
|            | 44           | QFQDYSNSR                          |
| RvY_04937  | 95           | NIPVGGVNTEATGDNYIR                 |
| RvY_06734  | 65           | AATGAVQSSASK                       |
|            | 51           | VLLDNQDDYELK                       |
| RvY_18401  | 42           | SGEQAMVDAK + Oxidation (M)         |
|            | 43           | SSTTTSTMNAAK                       |
|            | 54           | TTTTTGTGTGSGTTMPPK + Oxidation (M) |
| RvY_00840  | 38           | VVEQTGASSR                         |
|            | 65           | SSSSSSQESQSSSSQVR                  |
| RvY_12594  | 41           | NTSFNTTGDR                         |
|            | 51           | GYSYTNNTTYR                        |
| RvY_15001  | 45           | AVTGAVAGVK                         |
|            | 44           | QFQDYSNSR                          |
| RvY_04156  | 49           | GPEDEAGGPPK                        |
| RvY_04440  | 45           | SSGGNSPDVVVR                       |
| RvY_06311  | 42           | STVGQTPPQNLR                       |

**Supplementary Table 6.**  
**Statistical measures of DNA fragmentation in the alkaline comet assay**

| Experimental design                     | Trial ID | Dose  | Cell line | N <sup>a</sup> | % of DNA in Comet tail |       |
|---|----------|-------|-----------|----------------|------------------------|-------|
|   |          |       |           |                | Mean                   | s.d.  |
| Control<br>vs.<br>Dsup<br>vs.<br>DsupΔC | #1       | 0 Gy  | Control   | 302            | 0.25                   | 3.6   |
|   |          |       | Dsup      | 323            | 1.0                    | 2.1   |
|   |          |       | ΔC        | 252            | 0.94                   | 1.7   |
|   |          | 10 Gy | Control   | 281            | 33.8                   | 32.7  |
|   |          |       | Dsup      | 295            | 16.1                   | 9.7   |
|   |          |       | ΔC        | 298            | 37.2                   | 25.4  |
|   | #2       | 0 Gy  | Control   | 170            | 0.41                   | 1.4   |
|   |          |       | Dsup      | 173            | 0.017                  | 0.085 |
|   |          |       | ΔC        | 120            | 0.47                   | 1.8   |
|   |          | 10 Gy | Control   | 179            | 46.8                   | 44.6  |
|   |          |       | Dsup      | 202            | 23.4                   | 16.2  |
|   |          |       | ΔC        | 127            | 44.0                   | 49.0  |

<sup>a</sup> The number of analysed comets in each condition.

**Supplementary Table 7.**  
**Statistical test of the effect of Dsup on DNA fragmentation in the alkaline comet assay**

| Experimental design                     | Trial ID | Dose  | Comparison pair  | <i>p</i> -value       | Significance <sup>a</sup> |
|---|----------|-------|------------------|-----------------------|---------------------------|
|   |          |       |                  | (Tukey-Kramer's test) |                           |
| Control<br>vs.<br>Dsup<br>vs.<br>DsupΔC | #1       | 0 Gy  | Control vs. Dsup | 9.2E-05               | ***                       |
|   |          |       | Control vs. ΔC   | 0.0062                | **                        |
|   |          |       | Dsup vs. ΔC      | 0.94                  | n.s.                      |
|   |          | 10 Gy | Control vs. Dsup | 7.5E-13               | ***                       |
|   |          |       | Control vs. ΔC   | 0.22                  | n.s.                      |
|   |          |       | Dsup vs. ΔC      | 7.5E-13               | ***                       |
|   | #2       | 0 Gy  | Control vs. Dsup | 0.99                  | n.s.                      |
|   |          |       | Control vs. ΔC   | 0.53                  | n.s.                      |
|   |          |       | Dsup vs. ΔC      | 0.31                  | n.s.                      |
|   |          | 10 Gy | Control vs. Dsup | 1.6E-10               | ***                       |
|   |          |       | Control vs. ΔC   | 0.79                  | n.s.                      |
|   |          |       | Dsup vs. ΔC      | 1.7E-10               | ***                       |

<sup>a</sup> \*\**P*<0.01, \*\*\**P*<0.001, n.s. indicates not significant

**Supplementary Table 8.**

**Statistical measures of DNA damage induced by H<sub>2</sub>O<sub>2</sub> in the alkaline comet assay**

| Experimental design | Trial ID | Cell line | H <sub>2</sub> O <sub>2</sub><br>(100 µM) | NAC<br>(10 mM) | N <sup>a</sup> | % of DNA in Comet tail |      |
|---------------------|----------|-----------|---|----------------|----------------|------------------------|------|
|                     |          |           |   |                |                | Mean                   | s.d. |
| Control vs. Dsup    | #1       | Control   | -   | -              | 415            | 2.8                    | 5.5  |
|                     |          |           | +   | -              | 356            | 70.6                   | 26.1 |
|                     |          |           | +   | +              | 445            | 47.4                   | 30.3 |
|                     |          |           | -   | +              | 344            | 1.8                    | 2.9  |
|                     |          | Dsup      | -   | -              | 320            | 2.5                    | 6.1  |
|                     |          |           | +   | -              | 540            | 17.7                   | 12.6 |
|                     |          |           | +   | +              | 534            | 11.9                   | 10.1 |
|                     |          |           | -   | +              | 346            | 2.2                    | 3.1  |
|                     | #2       | Control   | -   | -              | 423            | 4.1                    | 5.0  |
|                     |          |           | +   | -              | 306            | 64.3                   | 31.7 |
|                     |          |           | +   | +              | 331            | 33.8                   | 32.4 |
|                     |          |           | -   | +              | 302            | 2.9                    | 4.1  |
|                     |          | Dsup      | -   | -              | 423            | 3.5                    | 6.5  |
|                     |          |           | +   | -              | 379            | 18.0                   | 15.6 |
|                     |          |           | +   | +              | 371            | 11.0                   | 8.3  |
|                     |          |           | -   | +              | 347            | 2.0                    | 2.7  |

<sup>a</sup> The number of analysed comets in each condition.

**Supplementary Table 9.**

**Statistical test of the effect of Dsup on damage by H<sub>2</sub>O<sub>2</sub> in the alkaline comet assay**

| Experimental design    | Trial ID | Comparison pair  | p-value<br>(Tukey-Kramer's test) | Significance <sup>a</sup> |
|------------------------|----------|--|----------------------------------|---------------------------|
| Control<br>vs.<br>Dsup | #1       | Control vs. Control+H <sub>2</sub> O <sub>2</sub>                                      | <1.0E-15                         | ***                       |
|                        |          | Control vs. Control+H <sub>2</sub> O <sub>2</sub> +NAC                                 | <1.0E-15                         | ***                       |
|                        |          | Control vs. Control+NAC  | 0.99                             | n.s.                      |
|                        |          | Control vs. Dsup   | 0.99                             | n.s.                      |
|                        |          | Control vs. Dsup+H <sub>2</sub> O <sub>2</sub>   | <1.0E-15                         | ***                       |
|                        |          | Control vs. Dsup+H <sub>2</sub> O <sub>2</sub> +NAC                                    | <1.0E-15                         | ***                       |
|                        |          | Control vs. Dsup+NAC   | 0.99                             | n.s.                      |
|                        |          | Control+H <sub>2</sub> O <sub>2</sub> vs. Control+H <sub>2</sub> O <sub>2</sub> +NAC   | <1.0E-15                         | ***                       |
|                        |          | Control+H <sub>2</sub> O <sub>2</sub> vs. Control+NAC                                  | <1.0E-15                         | ***                       |
|                        |          | Control+H <sub>2</sub> O <sub>2</sub> vs. Dsup   | <1.0E-15                         | ***                       |
|                        |          | Control+H <sub>2</sub> O <sub>2</sub> vs. Dsup+H <sub>2</sub> O <sub>2</sub>           | <1.0E-15                         | ***                       |
|                        |          | Control+H <sub>2</sub> O <sub>2</sub> vs. Dsup+H <sub>2</sub> O <sub>2</sub> +NAC      | <1.0E-15                         | ***                       |
|                        |          | Control+H <sub>2</sub> O <sub>2</sub> vs. Dsup+NAC                                     | <1.0E-15                         | ***                       |
|                        |          | Control+H <sub>2</sub> O <sub>2</sub> +NAC vs. Control+NAC                             | <1.0E-15                         | ***                       |
|                        |          | Control+H <sub>2</sub> O <sub>2</sub> +NAC vs. Dsup                                    | <1.0E-15                         | ***                       |
|                        |          | Control+H <sub>2</sub> O <sub>2</sub> +NAC vs. Dsup+H <sub>2</sub> O <sub>2</sub>      | <1.0E-15                         | ***                       |
|                        |          | Control+H <sub>2</sub> O <sub>2</sub> +NAC vs. Dsup+H <sub>2</sub> O <sub>2</sub> +NAC | <1.0E-15                         | ***                       |
|                        |          | Control+H <sub>2</sub> O <sub>2</sub> +NAC vs. Dsup+NAC                                | <1.0E-15                         | ***                       |
|                        |          | Control+NAC vs. Dsup   | 0.99                             | n.s.                      |
|                        |          | Control+NAC vs. Dsup+H <sub>2</sub> O <sub>2</sub>                                     | <1.0E-15                         | ***                       |
|                        |          | Control+NAC vs. Dsup+H <sub>2</sub> O <sub>2</sub> +NAC                                | <1.0E-15                         | ***                       |
|                        |          | Control+NAC vs. Dsup+NAC   | 0.99                             | n.s.                      |
|                        |          | Dsup vs. Dsup+H <sub>2</sub> O <sub>2</sub>  | <1.0E-15                         | ***                       |
|                        |          | Dsup vs. Dsup+H <sub>2</sub> O <sub>2</sub> +NAC                                       | <1.0E-15                         | ***                       |
|                        |          | Dsup vs. Dsup+NAC  | 0.99                             | n.s.                      |
|                        |          | Dsup+H <sub>2</sub> O <sub>2</sub> vs. Dsup+H <sub>2</sub> O <sub>2</sub> +NAC         | 2.86E-08                         | ***                       |
|                        |          | Dsup+H <sub>2</sub> O <sub>2</sub> vs. Dsup+NAC  | <1.0E-15                         | ***                       |
|                        |          | Dsup+H <sub>2</sub> O <sub>2</sub> +NAC vs. Dsup+NAC                                   | <1.0E-15                         | ***                       |
|                        | #2       | Control vs. Control+H <sub>2</sub> O <sub>2</sub>                                      | <1.0E-15                         | ***                       |
|                        |          | Control vs. Control+H <sub>2</sub> O <sub>2</sub> +NAC                                 | <1.0E-15                         | ***                       |
|                        |          | Control vs. Control+NAC  | 0.98                             | n.s.                      |
|                        |          | Control vs. Dsup   | 0.99                             | n.s.                      |
|                        |          | Control vs. Dsup+H <sub>2</sub> O <sub>2</sub>   | <1.0E-15                         | ***                       |
|                        |          | Control vs. Dsup+H <sub>2</sub> O <sub>2</sub> +NAC                                    | 9.45E-07                         | ***                       |
|                        |          | Control vs. Dsup+NAC   | 0.66                             | n.s.                      |
|                        |          | Control+H <sub>2</sub> O <sub>2</sub> vs. Control+H <sub>2</sub> O <sub>2</sub> +NAC   | <1.0E-15                         | ***                       |
|                        |          | Control+H <sub>2</sub> O <sub>2</sub> vs. Control+NAC                                  | <1.0E-15                         | ***                       |
|                        |          | Control+H <sub>2</sub> O <sub>2</sub> vs. Dsup   | <1.0E-15                         | ***                       |
|                        |          | Control+H <sub>2</sub> O <sub>2</sub> vs. Dsup+H <sub>2</sub> O <sub>2</sub>           | <1.0E-15                         | ***                       |
|                        |          | Control+H <sub>2</sub> O <sub>2</sub> vs. Dsup+H <sub>2</sub> O <sub>2</sub> +NAC      | <1.0E-15                         | ***                       |
|                        |          | Control+H <sub>2</sub> O <sub>2</sub> vs. Dsup+NAC                                     | <1.0E-15                         | ***                       |
|                        |          | Control+H <sub>2</sub> O <sub>2</sub> +NAC vs. Control+NAC                             | <1.0E-15                         | ***                       |
|                        |          | Control+H <sub>2</sub> O <sub>2</sub> +NAC vs. Dsup                                    | <1.0E-15                         | ***                       |
|                        |          | Control+H <sub>2</sub> O <sub>2</sub> +NAC vs. Dsup+H <sub>2</sub> O <sub>2</sub>      | <1.0E-15                         | ***                       |
|                        |          | Control+H <sub>2</sub> O <sub>2</sub> +NAC vs. Dsup+H <sub>2</sub> O <sub>2</sub> +NAC | <1.0E-15                         | ***                       |
|                        |          | Control+H <sub>2</sub> O <sub>2</sub> +NAC vs. Dsup+NAC                                | <1.0E-15                         | ***                       |
|                        |          | Control+NAC vs. Dsup   | 0.99                             | n.s.                      |
|                        |          | Control+NAC vs. Dsup+H <sub>2</sub> O <sub>2</sub>                                     | <1.0E-15                         | ***                       |
|                        |          | Control+NAC vs. Dsup+H <sub>2</sub> O <sub>2</sub> +NAC                                | 5.54E-08                         | ***                       |
|                        |          | Control+NAC vs. Dsup+NAC   | 0.99                             | n.s.                      |
|                        |          | Dsup vs. Dsup+H <sub>2</sub> O <sub>2</sub>  | <1.0E-15                         | ***                       |
|                        |          | Dsup vs. Dsup+H <sub>2</sub> O <sub>2</sub> +NAC                                       | 2.1E-08                          | ***                       |
|                        |          | Dsup vs. Dsup+NAC  | 0.95                             | n.s.                      |
|                        |          | Dsup+H <sub>2</sub> O <sub>2</sub> vs. Dsup+H <sub>2</sub> O <sub>2</sub> +NAC         | 2.3E-08                          | ***                       |
|                        |          | Dsup+H <sub>2</sub> O <sub>2</sub> vs. Dsup+NAC  | <1.0E-15                         | ***                       |
|                        |          | Dsup+H <sub>2</sub> O <sub>2</sub> +NAC vs. Dsup+NAC                                   | 1.23E-10                         | ***                       |

<sup>a</sup> \*\*\**P*<0.001, n.s. indicates not significant

**Supplementary Table 10.**

**Statistical measures of DNA fragmentation in the neutral comet assay**

| Experimental design | Trial ID | Dose | Cell line | N <sup>a</sup> | % of DNA in Comet tail |      |
|---------------------|----------|------|-----------|----------------|------------------------|------|
|                     |          |      |           |                | Mean                   | s.d. |
| Control vs. Dsup    | #1       | 0 Gy | Control   | 300            | 7.5                    | 8.7  |
|                     |          |      | Dsup      | 300            | 5.8                    | 6.5  |
|                     |          | 5 Gy | Control   | 300            | 35.5                   | 26.3 |
|                     |          |      | Dsup      | 300            | 21.1                   | 21.0 |
|                     | #2       | 0 Gy | Control   | 300            | 11.7                   | 6.7  |
|                     |          |      | Dsup      | 300            | 13.0                   | 6.8  |
|                     |          | 5 Gy | Control   | 300            | 30.5                   | 10.2 |
|                     |          |      | Dsup      | 300            | 20.4                   | 9.7  |
|                     | #3       | 0 Gy | Control   | 300            | 9.6                    | 11.9 |
|                     |          |      | Dsup      | 300            | 10.9                   | 7.0  |
|                     |          | 5 Gy | Control   | 300            | 32.7                   | 12.1 |
|                     |          |      | Dsup      | 300            | 19.0                   | 10.0 |

<sup>a</sup> The number of analysed comets in each condition.

**Supplementary Table 11.**

**Pairwise statistical test of the effect of Dsup on DNA fragmentation in the neutral comet assay**

| Pairwise comparison | Trial ID | Dose | <i>p</i> -value (F-test) <sup>a</sup> | Statistical method       | <i>t</i> -value | <i>p</i> -value | Significance <sup>b</sup> |
|---------------------|----------|------|---------------------------------------|--------------------------|-----------------|-----------------|---------------------------|
| Control vs. Dsup    | #1       | 0 Gy | 7.8E-07                               | Welch's <i>t</i> -test   | 2.758           | 0.0060          | **                        |
|                     |          | 5 Gy | 8.9E-05                               | Welch's <i>t</i> -test   | 7.406           | 4.7E-13         | ***                       |
|                     | #2       | 0 Gy | 8.8E-01                               | Student's <i>t</i> -test | -2.266          | 0.024           | *                         |
|                     |          | 5 Gy | 3.4E-01                               | Student's <i>t</i> -test | 6.263           | 7.2E-10         | ***                       |
|                     | #3       | 0 Gy | 2.8E-18                               | Welch's <i>t</i> -test   | -1.541          | 0.12            | n.s.                      |
|                     |          | 5 Gy | 6.0E-04                               | Welch's <i>t</i> -test   | 15.099          | <1.0E-15        | ***                       |

<sup>a</sup> Equality of variances was tested using F-test (significance level = 0.05).

<sup>b</sup> \**P*<0.05, \*\**P*<0.01, \*\*\**P*<0.001, n.s. indicates not significant

**Supplementary Table 12.**

**Statistical data regarding the number of foci in the  $\gamma$ -H2AX assay**

| Experimental design                              | Trial ID | Dose | Cell line   | N <sup>a</sup> | The number of foci / nucleus |      |
|--|----------|------|-------------|----------------|------------------------------|------|
|  |          |      |             |                | Mean                         | s.d. |
| Control vs. Dsup                                 | #1       | 0 Gy | Control     | 52             | 11.0                         | 13.7 |
|  |          |      | Dsup        | 73             | 6.8                          | 8.9  |
|  |          | 1 Gy | Control     | 53             | 38.4                         | 15.0 |
|  |          |      | Dsup        | 71             | 22.8                         | 11.8 |
| Control<br>vs.<br>Dsup<br>vs.<br>Dsup+shDsup     | #1       | 0 Gy | Control     | 77             | 3.2                          | 1.9  |
|  |          |      | Dsup        | 74             | 5.2                          | 2.0  |
|  |          |      | Dsup+shDsup | 70             | 3.2                          | 1.2  |
|  |          | 1 Gy | Control     | 70             | 40.1                         | 9.1  |
|  |          |      | Dsup        | 92             | 26.6                         | 5.2  |
|  |          |      | Dsup+shDsup | 74             | 42.1                         | 5.9  |
|  | #2       | 0 Gy | Control     | 40             | 3.8                          | 4.2  |
|  |          |      | Dsup        | 35             | 5.9                          | 3.4  |
|  |          |      | Dsup+shDsup | 40             | 4.8                          | 3.8  |
|  |          | 1 Gy | Control     | 36             | 38.5                         | 5.2  |
|  |          |      | Dsup        | 54             | 28.0                         | 2.1  |
|  |          |      | Dsup+shDsup | 40             | 38.4                         | 4.4  |
| Control<br>vs.<br>Dsup<br>vs.<br>Dsup $\Delta$ C | #1       | 0 Gy | Control     | 59             | 0.19                         | 0.58 |
|  |          |      | Dsup        | 63             | 1.0                          | 0.22 |
|  |          |      | $\Delta$ C  | 62             | 0.32                         | 0.27 |
|  |          | 1 Gy | Control     | 59             | 28.4                         | 5.1  |
|  |          |      | Dsup        | 74             | 14.8                         | 6.4  |
|  |          |      | $\Delta$ C  | 60             | 22.2                         | 7.5  |
|  | #2       | 0 Gy | Control     | 77             | 5.4                          | 3.4  |
|  |          |      | Dsup        | 65             | 7.9                          | 1.0  |
|  |          |      | $\Delta$ C  | 62             | 3.9                          | 2.7  |
|  |          | 1 Gy | Control     | 75             | 50.6                         | 7.5  |
|  |          |      | Dsup        | 64             | 33.8                         | 1.5  |
|  |          |      | $\Delta$ C  | 73             | 46.7                         | 7.1  |

<sup>a</sup> The number of analysed nuclei in each condition.



**Supplementary Table 13.**

**Pairwise statistical test of the effect of Dsup on the number of foci in the  $\gamma$ -H2AX assay**

| Pairwise comparison | Trial ID | Dose | <i>p</i> -value (F-test) <sup>a</sup> | Statistical method     | <i>t</i> -value | <i>p</i> -value | Significance <sup>b</sup> |
|---------------------|----------|------|---------------------------------------|------------------------|-----------------|-----------------|---------------------------|
| Control vs. Dsup    | #1       | 0 Gy | 7.84.E-04                             | Welch's <i>t</i> -test | 1.962           | 0.052           | n.s.                      |
|                     |          | 1 Gy | 5.90.E-02                             | Welch's <i>t</i> -test | 6.489           | 3.8E-09         | ***                       |

<sup>a</sup> Equality of variances was tested using F-test (significance level = 0.05).

<sup>b</sup> \*\*\**P*<0.001, n.s. indicates not significant

**Supplementary Table 14.**

**Statistical analysis of the effect of Dsup on the number of foci in the  $\gamma$ -H2AX assay**

| Experimental design              | Trial ID | Dose | Comparison pair         | <i>p</i> -value (Tukey-Kramer's test) | Significance <sup>a</sup> |
|----------------------------------|----------|------|-------------------------|---------------------------------------|---------------------------|
| Control vs. Dsup vs. Dsup+shDsup | #1       | 0 Gy | Control vs. Dsup        | 0.15                                  | n.s.                      |
|                                  |          |      | Control vs. Dsup+shDsup | 1.0                                   | n.s.                      |
|                                  |          |      | Dsup vs. Dsup+shDsup    | 0.15                                  | n.s.                      |
|                                  |          | 1 Gy | Control vs. Dsup        | 0.0023                                | **                        |
|                                  |          |      | Control vs. Dsup+shDsup | 0.92                                  | n.s.                      |
|                                  |          |      | Dsup vs. Dsup+shDsup    | 8.9E-04                               | ***                       |
|                                  | #2       | 0 Gy | Control vs. Dsup        | 0.70                                  | n.s.                      |
|                                  |          |      | Control vs. Dsup+shDsup | 0.90                                  | n.s.                      |
|                                  |          |      | Dsup vs. Dsup+shDsup    | 0.89                                  | n.s.                      |
|                                  |          | 1 Gy | Control vs. Dsup        | 0.0094                                | **                        |
|                                  |          |      | Control vs. Dsup+shDsup | 1.0                                   | n.s.                      |
|                                  |          |      | Dsup vs. Dsup+shDsup    | 0.0036                                | **                        |
| Control vs. Dsup vs. DsupΔC      | #1       | 0 Gy | Control vs. Dsup        | 0.38                                  | n.s.                      |
|                                  |          |      | Control vs. ΔC          | 0.69                                  | n.s.                      |
|                                  |          |      | Dsup vs. ΔC             | 0.14                                  | n.s.                      |
|                                  |          | 1 Gy | Control vs. Dsup        | 0.0039                                | **                        |
|                                  |          |      | Control vs. ΔC          | 0.57                                  | n.s.                      |
|                                  |          |      | Dsup vs. ΔC             | 0.027                                 | *                         |
|                                  | #2       | 0 Gy | Control vs. Dsup        | 0.018                                 | *                         |
|                                  |          |      | Control vs. ΔC          | 0.89                                  | n.s.                      |
|                                  |          |      | Dsup vs. ΔC             | 0.047                                 | *                         |
|                                  |          | 1 Gy | Control vs. Dsup        | 0.0022                                | **                        |
|                                  |          |      | Control vs. ΔC          | 0.14                                  | n.s.                      |
|                                  |          |      | Dsup vs. ΔC             | 0.17                                  | n.s.                      |

<sup>a</sup> \**P*<0.05, \*\**P*<0.01, \*\*\**P*<0.001, n.s. indicates not significant

**Supplementary Table 15. Effect of Dsup on the cell number over time**

| Experimental design              | Trial ID | Dose | Cell line   | N | 8 dps             |                   | 10 dps            |                   | 12 dps            |                   |
|----------------------------------|----------|------|-------------|---|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
|                                  |          |      |             |   | Mean <sup>a</sup> | s.d. <sup>a</sup> | Mean <sup>a</sup> | s.d. <sup>a</sup> | Mean <sup>a</sup> | s.d. <sup>a</sup> |
| Control vs. Dsup vs. Dsup+shDsup | #1       | 0 Gy | Control     | 3 | 44,502            | 9,694             | 117,649           | 37,205            | 179,154           | 23,127            |
|                                  |          |      | Dsup        | 3 | 219,804           | 60,518            | 618,684           | 37,335            | 733,030           | 78,866            |
|                                  |          |      | Dsup+shDsup | 3 | 24,725            | 3,778             | 75,734            | 29,838            | 182,584           | 10,981            |
|                                  |          | 4 Gy | Control     | 3 | 1,264             | 484               | 931               | 74                | 343               | 388               |
|                                  |          |      | Dsup        | 3 | 5,772             | 812               | 18,914            | 6,556             | 82,487            | 27,745            |
|                                  |          |      | Dsup+shDsup | 3 | 1,441             | 473               | 1,686             | 450               | 882               | 574               |
|                                  | #2       | 0 Gy | Control     | 3 | 19,963            | 3,602             | 43,218            | 6,285             | 165,992           | 32,313            |
|                                  |          |      | Dsup        | 3 | 151,273           | 33,213            | 600,975           | 54,620            | 826,914           | 53,068            |
|                                  |          |      | Dsup+shDsup | 3 | 42,463            | 13,143            | 114,895           | 60,949            | 193,981           | 54,863            |
|                                  |          | 4 Gy | Control     | 3 | 1,392             | 309               | 8,840             | 4,164             | 539               | 279               |
|                                  |          |      | Dsup        | 3 | 3,440             | 841               | 1,166             | 767               | 54,606            | 7,044             |
|                                  |          |      | Dsup+shDsup | 3 | 1,156             | 456               | 1,245             | 1,095             | 510               | 139               |
| Control vs. Dsup vs. DsupΔC      | #1       | 0 Gy | Control     | 3 | 22,072            | 10,508            | 51,120            | 9,477             | 256,735           | 54,945            |
|                                  |          |      | Dsup        | 3 | 29,021            | 5,603             | 92,071            | 7,911             | 418,379           | 52,428            |
|                                  |          |      | ΔC          | 3 | 18,840            | 2,143             | 46,433            | 7,335             | 335,071           | 20,356            |
|                                  |          | 4 Gy | Control     | 3 | 1,481             | 584               | 1,899             | 1,698             | 1,205             | 1,615             |
|                                  |          |      | Dsup        | 3 | 4,888             | 1,603             | 22,261            | 2,485             | 60,627            | 5,739             |
|                                  |          |      | ΔC          | 3 | 3,232             | 450               | 7,979             | 1,296             | 8,470             | 2,583             |
|                                  | #2       | 0 Gy | Control     | 3 | 42,555            | 9,085             | 106,635           | 13,435            | 646,104           | 51,957            |
|                                  |          |      | Dsup        | 3 | 58,445            | 22,937            | 188,612           | 4,595             | 985,464           | 171,824           |
|                                  |          |      | ΔC          | 3 | 46,931            | 3,498             | 138,450           | 18,236            | 756,584           | 28,496            |
|                                  |          | 4 Gy | Control     | 3 | 4,134             | 790               | 8,262             | 2,308             | 8,471             | 3,336             |
|                                  |          |      | Dsup        | 3 | 9,211             | 2,359             | 24,179            | 5,348             | 63,105            | 14,288            |
|                                  |          |      | ΔC          | 3 | 6,801             | 993               | 13,069            | 3,455             | 17,922            | 6,023             |

<sup>a</sup> Mean and standard deviation (s.d.) values are shown for the cell number at each point.  
dps; days post seeding

## **Supplementary Methods**

### **Hygienic rearing of the tardigrades**

The YOKOZUNA-1 strain of the tardigrade *R. varieornatus* were reared on water-layered agar plates by feeding them alga, *Chlorella vulgaris* enriched with vitamin B12 (Chlorella Industry, Japan), at 22°C. To minimize bacterial contamination in tardigrade culture, tardigrade eggs were occasionally collected and their surfaces were cleansed by treatment with 1:100 diluted commercial chlorine bleach containing sodium hypochlorite for several minutes. After several washes with sterilized MilliQ water (Millipore), the treated eggs were placed in fresh culture. To examine elimination of bacteria, some treated eggs were cultured in LB medium with shaking overnight and we observed no apparent bacterial crowding in the medium. Note: We used the purchased live chlorella suspension (Chlorella Industry, Japan) as a food. The suspension was not axenic and thus our tardigrade culture is also not free from bacteria.

### **Preparation of tardigrades for genome DNA extraction**

We used adult tardigrades grown from the hypochlorite-treated eggs. Tardigrades were transferred to fresh agar plates overlaid with sterilized MilliQ water. Most tardigrades clung to the agar surface with their claws, and were efficiently washed with water flow by pipetting. Bacterial contaminants on their claws were wiped off while the tardigrades walked on the clean agar surface. Tardigrades were starved for 2 d to facilitate digestion of food (algae) in their gut, and treated with antibiotics, penicillin-streptomycin solution (Invitrogen, 15140-122), to suppress bacterial growth. Water and antibiotics were replaced daily. After 2 d starvation and antibiotic treatment, tardigrades were extensively washed with sterilized MilliQ water, and subjected to genome DNA extraction.

### **Preparation of tardigrades for total RNA extraction**

Adult tardigrades were starved for 2 d to remove the food in the gut. Antibiotic treatment was omitted to avoid their possible effects on gene expression and instead the animals were extensively washed after starvation. To induce anhydrobiosis, the tardigrades were dropped onto a nylon mesh filter (pore size 11 µm; 25 mm in diameter;

Millipore) placed on 3MM filter paper (25 mm in diameter; Whatman) and then desiccated for 2 d in a tightly sealed plastic container, in which the relative humidity was regulated at 33.8% with 83% (w/w) glycerol in water solution. For rehydration, dehydrated tardigrades on the mesh were placed in a thin water layer in a fresh agar dish and rehydrated tardigrades were collected in 1.5-mL tubes at 80 min or 3 h after rehydration. Excess water was removed after short centrifugation and the tardigrade pellet was disrupted using a plastic pestle in TRIzol reagent (Invitrogen) and total RNA was extracted according to manufacturer's instructions. Dehydrated tardigrades were collected by placing the closely trimmed mesh containing the tardigrades into 1.5-mL tubes. Addition of TRIzol reagent solubilized the nylon mesh and total RNA was extracted similarly as for the hydrated tardigrades. Embryos were collected with 2-d intervals after egg laying and subjected to RNA extraction after extensive washes without hypochlorite treatment.

### **Feulgen Densitometry**

All procedures were performed at room temperature. An adult tardigrade in a drop of water was placed on a slide glass and squashed to obtain single cells. The slide was air dried and fixed in MFA (methanol, formalin, acetic acid = 85:10:5 v/v) for 24 h, hydrolysed in 5.0 N HCl for 120 min, and stained using Schiff reagent for 120 min. The images of stained tardigrade cells were captured with a BX-51 microscope and DP-50 CCD camera (Olympus, Osaka, Japan). The linearity of the camera response was tested prior to the measurements by using a stepped density filter (Edmund Optics, Barrington, NJ). The green channel was extracted from images, and the density of Feulgen stain in the nuclei was measured using image analysis software, FMBIO Analysis (Hitachi Software, Tokyo, Japan). In total, 119 cells from 10 animals were examined. The genome size of *R. varieornatus* was normalized by the C-value of stained cells obtained from *D. melanogaster*.